4th European conference on Xylella fastidiosa 2023

Researching sustainable solutions

19 – 20 August 2023, Lyon (France)

Book of Abstracts

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Introduction

The 4th European conference on *Xylella fastidiosa* is held on 20 August 2023 in Lyon (France), as a Satellite event of the International Congress on Plant Pathology (ICPP2023). It brings together researchers, risk assessors, risk managers and stakeholders to discuss the results from research that has taken place since *X. fastidiosa* was first detected in Europe in 2013.

Organised by the European Food Safety Authority (EFSA) with the active contribution of the major EU and transnational research initiatives on *X. fastidiosa* (BeXyl, BIOVEXO, ERC MultiX and EUPHRESCO), the conference is chaired by Blanca B. Landa, who coordinates the EU funded project BeXyl (*Beyond Xylella*), and Giuseppe Stancanelli, who leads the Plant Health Risk Assessment team at EFSA. The Scientific Committee of this conference includes scientists from all the partner organisations and external reviewers.

The presentations and discussions take stock of the results from research that has taken place over the last decade. Two major research projects (PonTE and XF-ACTORS) funded by the European Union have filled knowledge gaps on the distinctive elements of the outbreaks, consolidated a robust network for monitoring and testing, and produced results that have supported and influenced the activities of researchers, stakeholders and policy makers.

The programme reflects the progress of the multidisciplinary research conducted and discuss the scientific approaches to develop and test practical and applicable solutions for the control and sustainable management of *X. fastidiosa* outbreaks in the EU.

The conference sessions and discussions cover the latest findings, data and knowledge on *X. fastidiosa*, including:

- Pathogen biology, ecology and genetics;
- Epidemiology and modelling of *X. fastidiosa* diseases;
- Host plant-interactions and resistant/tolerant plant germplasm;
- Surveillance and early detection tools;
- Vectors biology and sustainable control strategies;
- Endophytic microbial resources for *X. fastidiosa* control;
- Sustainable and integrated pest and disease management strategies for prevention and reduction of the impact of *X. fastidiosa* diseases.

Young researchers' initiative

The Young Researchers' Initiative is designed to support early-career researchers and plant health professionals, by covering accommodation and travel costs to attend the conference.

EFSA has selected 13 researchers for this initiative, hailing from Europe, America, and Asia. To be considered, participants needed to be a presenter of an accepted oral or poster presentation, be either a PhD student or an early career researcher/plant health professional, and be affiliated with a public institute or higher education institute.

The young researchers selected are:

- **Manuel Anguita-Maeso, speaker** (University of North Carolina, United States, and Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain)
- **Renata Assis, speaker** (University of California (UC) - Davis, United States)
- **Nicola Bodino, speaker** (Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR), Italy)
- **Franco Fernandez, poster presenter** (Instituto de Patología Vegetal, Centro de Investigaciones Agropecuarias, Instituto Nacional de Tecnología Agropecuaria, Argentina)
- **Nancy Her, speaker** (University of California (UC) - Riverside, United States)
- **Hysen Kokiçi, poster presenter** (Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR), Italy)
- **Leticia Lucero, poster presenter** (National Center for Biotechnology (CNB) of the Spanish National Research Council (CSIC), Spain)
- **Lena Pesenti, speaker and poster presenter** (Université Catholique de Louvain (UCLouvain), Belgium)
- **Isabel Rodrigues, poster presenter** (Centro de Investigação de Montanha (CIMO) and Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Portugal)
- **Miguel Román-Écija, speaker and poster presenter** (Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain)
- **Antony Surano, poster presenter** (Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR), Italy)
- **Miri Vanunu, poster presenter** (Department of Plant Pathology and Weed Research, Agricultural Research Organization, Volcani Institute, Israel, and The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel)
- **Maria del Pilar Velasco-Amo, speaker** (Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain)

Scientific committee

- **Claude Bragard** (Chair of EFSA Panel on Plant Health, Université Catholique de Louvain (UCLouvain), Belgium)

- **Stephane Compant** (BIOVEXO project, AIT Austrian Institute of Technology, Austria)

- **Astrid Cruaud** (National Institute for Agriculture, Food and the Environment (INRAE), France)

- **Alice Delbianco** (European Food Safety Authority (EFSA))

- **Baldissera Giovani** (Euphresco network)

- **Blanca B. Landa** (BeXyl project coordinator, Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain)

- **Silke Robatzek** (MultiX project, Ludwig-Maximilians-Universitaet Muenchen (LMU), Germany)

- **Giuseppe Stancanelli** (European Food Safety Authority (EFSA))

Conference programme

Venue: Lyon Convention Centre, 50 quai Charles de Gaulle – 69006 Lyon (FR)

Saturday 19th August 2023

18.00-20.00 Registration and posters hanging

18.30-20.00	**Welcome and networking reception of the 4th European conference on *Xylella fastidiosa***
20.00-21.15	**Screening of the documentary movie on *Xylella fastidiosa*** **"IL TEMPO DEI GIGANTI"** ("THE ERA OF GIANTS", in Italian with English subtitles) a film by Davide Barletti and Lorenzo Conte, produced by Dinamo Film and Fluid Produzioni, distributed internationally by Summerside Media

Sunday 20th August 2023

08.00-09.00 Registration and posters hanging

SESSION 1 – Current status and research updates on *Xylella fastidiosa* in the EU **Chairs: Blanca B. Landa** (BeXyl project coordinator, Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain) and **Giuseppe Stancanelli** (European Food Safety Authority (EFSA))		
09.00-09.10	Welcome and brief introduction to the conference	
09.10-09.20	Current pest status of *X. fastidiosa* in the European Union	**Maria Belen Marquez Garcia** Directorate General for Health and Food Safety (DG SANTE), European Commission
09.20-09.30	The current status of *X. fastidiosa* in Salento (Italy): are we approaching a new phase of the epidemic?	**Donato Boscia** Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR), Italy
09.30-09.40	Comparative genomics of *X. fastidiosa* subsp. *multiplex* strains from France reveals pathogen dynamics after its introduction	**Jessica Dittmer** National Institute for Agriculture, Food and the Environment (INRAE), France

09.40-09.50	*X. fastidiosa* infection alters the xylem microbiota in wild and cultivated olive trees	**Manuel Anguita-Maeso*** University of North Carolina, United States, and Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain **EFSA Young Researchers' Initiative*
09.50-10.00	What is the risk of *X. fastidiosa* to establish in temperate European regions - a Belgian case study	**Lena Pesenti*** Université Catholique de Louvain (UCLouvain), Belgium **EFSA Young Researchers' Initiative*
10.00-10.30	Q&A and discussion	
10.30-11.00	Coffee/Tea break	
SESSION 2 – Latest findings on *Xylella fastidiosa* resistance and control **Chairs: Stéphane Compant** (AIT Austrian Institute of Technology, Austria) and **Maria Saponari** (Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR), Italy)		
11.00-11.10	How many plant species can *X. fastidiosa* infect? An update from the EFSA host plants database	**Giuseppe Stancanelli** European Food Safety Authority (EFSA)
11.10-11.20	Identification and characterization of a *X. fastidiosa* resistant almond genotype	**Ofir Bahar** Agricultural Research Organization (ARO), Volcani Center, Israel
11.20-11.30	Enhancing rootstock-mediated systemic immunity against Pierce's Disease in grafted Chardonnay	**Renata Assis*** University of California (UC) - Davis, United States **EFSA Young Researchers' Initiative*
11.30-11.40	Novel target-oriented peptides with potential for infection control strategies against *X. fastidiosa*	**Luis Alejandro Moll dos Santos** Institute of Food and Agricultural Technology (INTEA), University of Girona (UdG), Spain
11.40-11.50	Grapevines primed with lipopolysaccharide demonstrates systemic resistance to Pierce's disease and reveals an important peroxidase mechanism linked to the immune memory	**Nancy Her*** University of California (UC) - Riverside, United States **EFSA Young Researchers' Initiative*

11.50-12.00	High prevalence of resistant genotypes to *X. fastidiosa* in natural olive resources derived from the cultivar Leccino	**Pasquale Saldarelli** Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR), Italy
12.00-12.30	Q&A and discussion	
12.30-14.30 *12.40-13.30* *13.30-14.20*	Lunch break and POSTERS SESSION *Presentation of posters with odd numbers* *Presentation of posters with even numbers*	
SESSION 3 - *Xylella fastidiosa* vectors biology **Chairs: Rodrigo Almeida** (University of California (UC) - Berkeley, United States) and **Astrid Cruaud** (National Institute for Agriculture, Food and the Environment (INRAE), France)		
14.30-14.40	Reproductive biology and egg parasitoids of *Philaenus spumarius*	**Nicola Bodino*** Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR), Italy **EFSA Young Researchers' Initiative*
14.40-14.50	Factors driving insect vector presence, abundance and pathogen transmission: the case of *P. spumarius* and *Neophilaenus campestris*	**Júlia López-Mercadal** Universitat de les Illes Balears (UIB), Spain
14.50-15.00	Ecological aspects of spittlebugs of the genus *Clastoptera* (Hemiptera: Cercopoidea: Clastopteridade), possible vectors of *X. fastidiosa* in olives in southeastern Brazil	**João R.S. Lopes** College of Agriculture Luiz De Queiroz (ESALQ), University of Sao Paulo (USP), Brasil
15.00-15.10	Ability of glassy-winged sharpshooter to acquire *X. fastidiosa* subsp. *pauca* from ripe olive varieties grown in California, USA	**Lindsey Burbank** US Department of Agriculture (USDA), Agricultural Research Service (ARS), United States
15.10-15.20	Degree-day-based model to predict egg hatching of *P. spumarius* (Hemiptera: Aphrophoridae), the main vector of *X. fastidiosa* in Europe	**Alberto Fereres** Institute of Agricultural Sciences (ICA) of the Spanish National Research Council (CSIC), Spain
15.20-15.30	Vectors as sentinels of plant diseases in a changing world: rising temperatures increase the risk of *X. fastidiosa* outbreaks	**Astrid Cruaud** National Institute for Agriculture, Food and the Environment (INRAE), France
15.30-16.00	Q&A and discussion	
16.00-16.30	Coffee/Tea break	

SESSION 4 - Tools for *Xylella fastidiosa* surveillance and detection **Chairs: Claude Bragard** (Chair of EFSA Panel on Plant Health, Université Catholique de Louvain (UCLouvain), Belgium) and **Valérie Grimault** (European and Mediterranean Plant Protection Organization (EPPO))		
16.30-16.40	Use of plasmid profiles in epidemiological surveillance of *X. fastidiosa* outbreak in the Valencian Community, Spain	**Maria del Pilar Velasco-Amo*** Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain **EFSA Young Researchers' Initiative*
16.40-16.50	Early detection of *X. fastidiosa* infection using reflectance proximal sensors in almond and olive trees	**Miguel Román-Écija*** Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain **EFSA Young Researchers' Initiative*
16.50-17.00	Detection of symptoms induced by *X. fastidiosa* with high-resolution multispectral satellite data: assessment with airborne hyperspectral imagery	**Tomas Poblete** University of Melbourne, Australia
17.00-17.10	RiPEST: EFSA develops a tool for designing more robust and harmonized pest surveys in the EU	**Alice Delbianco** European Food Safety Authority (EFSA)
17.10-17.20	Delimiting survey of *X. fastidiosa* subsp. *multiplex*: bringing together multidisciplinary expertise to support risk management	**Pauline De Jerphanion** French General Directorate for Food (DGAL), French Ministry of Agriculture, and French Epidemiological Plant Health Surveillance Platform, France
17.20-17.30	Evaluation of outbreak response plans for *X. fastidiosa* in Alicante, Spain	**Martina Cendoya** Valencian Institute for Agricultural Research (IVIA), Spain
17.30-18.00	Q&A and discussion	
CLOSING REMARKS **Chairs: Blanca B. Landa** (BeXyl project coordinator, Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain) and **Giuseppe Stancanelli** (European Food Safety Authority (EFSA))		
18.00-18.30	Closing remarks and wrap-up	

Oral presentations

SESSION 1 – Current status and research updates on *Xylella fastidiosa* in the EU

CURRENT PEST STATUS OF *XYLELLA FASTIDIOSA* IN THE EUROPEAN UNION

Marquez Garcia M.B. (1)

(1) Directorate General for Health and Food Safety (DG SANTE), European Commission

Xylella fastidiosa is a Union quarantine pest known to be present in the EU territory (Commission Implementing Regulation (EU) 2019/2072) and it is also listed as a priority pest (Commission Delegated Regulation (EU) 2019/1702).

Since the first finding of *X. fastidiosa* in the EU in 2013, the Commission put in place specific measures to control this pest and updated them following the scientific developments. Today, the measures against the spread and introduction into the EU of *X. fastidiosa* are detailed in Commission Implementing Regulation (EU) 2020/1201. Prevention and early detection are key pillars in the fight against this pest. The measures detail the general requirements for surveys in the EU territory, the eradication and containment measures for those areas where the pest is known to be present, the movement of specified plant within the Union territory and the requirements for the introduction of host plants from third countries.

Based on the annual survey campaigns in all Member States, *X. fastidiosa* is known as absent in 23 Member States and present in 4 Member States where the affected areas have been demarcated for the control of the pest.

The most recent update of the list of host plants was voted in July 2023.

THE CURRENT STATUS OF *XYLELLA FASTIDIOSA* IN SALENTO (ITALY): ARE WE APPROACHING A NEW PHASE OF THE EPIDEMIC?

Boscia D. (1), Cavalieri V. (1), Dongiovanni C. (2), Giampetruzzi A. (1), Saldarelli P. (1), Saponari M. (1)

(1) Institute for Sustainable Plant Protection - National Research Council, Bari, ITALY; (2) Centro Ricerca Formazione e Sperimentazione in Agricoltura "Basile Caramia", Locorotondo, Bari, ITALY

During these 10 years from the emergence of *Xylella fastidiosa* in olive trees in Salento, the destructive potential of its epidemic spread and the consequent dramatic impacts on several agroecosystem services, have been extensively documented. Main part of the territory of this peninsula has been included in the "demarcated infected area", where several containment measures are strongly recommended, and few are the mandatory (i.e. banning plantations of highly susceptible species). Such status coupled with favorable epidemiological conditions caused the persistence of the infections in the whole area, decimating millions of olive trees, the main crop and landscape species of the area. Most likely, the wide occurrence of deadly infections entails a reduction of the reservoirs of bacterial inoculum and infective insect vectors. Alongside, in the last couple of years an increasing number of occasional reports of symptoms remittance in trees not severely affected has been recorded, posing new complex questions to the scientific community. Initial investigations on recently isolated strains seem to exclude the insurgence of adaptive traits and loss of aggressiveness. While preliminary data on the evolution of the vector infectivity showed a decreasing trend; probably resulting in a reduction of the superinfections events on the surviving trees. New studies are therefore now necessary to understand the factors driving this new stage of the epidemic.

COMPARATIVE GENOMICS OF *XYLELLA FASTIDIOSA* SUBSP. *MULTIPLEX* STRAINS FROM FRANCE REVEALS PATHOGEN DYNAMICS AFTER ITS INTRODUCTION

Dittmer J. (1), Briand M. (1), Legendre B. (2), Cunty A. (2), Boutigny A. (2), Grilloton K. (1), Bahut M. (3), Cesbron S. (1), Jacques M. (1)

(1) INRAE, Beaucouzé, FRANCE; (2) French Agency for Food, Environmental and Occupational Health & Safety, Plant Health Laboratory, Angers, FRANCE; (3) Université d'Angers, Angers, FRANCE

Xylella fastidiosa is a plant pathogen responsible for numerous crop diseases worldwide. It specifically colonizes the xylem of plants and is transmitted exclusively by sap-feeding insects. *X. fastidiosa* has a significant adaptive capacity, as evidenced by its great genetic diversity and frequent recombination events between subspecies. Originating from the Americas, X. fastidiosa is now present in several European countries (Italy, France, Spain and Portugal) due to accidental introductions of contaminated plant material. This situation calls for a better understanding of the evolutionary dynamics of the pathogen in its new areas of distribution. Herein, we present a comparative genomics analysis of strains belonging to the subspecies multiplex that have been isolated from various host plants in France since the first detection of the pathogen in 2015. High-quality genome sequences were obtained using the PacBio sequencing technology, which allowed the identification of numerous plasmids, in contrast to American strains of this subspecies which rarely possess plasmids. We further employed a metagenomic sequencing approach combining both long- and short-read technologies to sequence additional genomes directly from infected plants in a new focus of infection. This strategy simplifies the sequencing of new strains detected in the field by eliminating the time-consuming isolation step, thereby providing access to strains that could not have been sequenced previously.

XYLELLA FASTIDIOSA INFECTION ALTERS THE XYLEM MICROBIOTA IN WILD AND CULTIVATED OLIVE TREES

Anguita-Maeso M. (1,2)*, Belaj A. (3), Olmo D. (4), Navas-Cortés J.A. (2), Landa B.B. (2)

(1) University of North Carolina at Chapel Hill, Chapel Hill, UNITED STATES; (2) Institute for Sustainable Agriculture, Spanish National Research Council (IAS-CSIC), Córdoba, SPAIN; (3) Centro Alameda del Obispo, Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA), Córdoba, SPAIN; (4) Institute of Agro-Food and Fisheries Research and Training of the Balearic Islands (IRFAP), Palma de Mallorca, SPAIN

**EFSA Young Researchers' Initiative*

Nowadays, there are no tools available to cure *Xylella fastidiosa* (Xf) once a plant becomes infected. Research on plant-associated microorganisms is gaining increasing interest as an innate natural defense of plants to cope against infection by pathogens. In this study we have characterized the xylem-inhabiting bacterial communities in olive trees infected or not by Xf under natural field conditions in the Balearic Islands (Spain) by using a NGS approach. We have also evaluated the potential differences in the xylem microbiota associated to wild and cultivated (native or introduced) olive genotypes. For that, 244 samples from leaf petioles of *Olea europaea* plants were sampled and fingerprinted using an EST-SNP marker analysis to identify the olive accession. Alpha and beta-diversity measures of bacterial communities indicated that the main significant differences were associated to Xf infection, followed by the origin of the olive genotype (wild or cultivated) within each island (Mallorca, Menorca and Ibiza). Also, we have identified different bacterial genera significantly enriched according to Xf infection and olive genotype. The relationship between the genetic closeness of cultivated versus wild olive genotypes along with their differences in the xylem microbiome will be addressed.

Supported by Projects PID2020-114917RB-I00 and TED2021-130110B (AEI-Spain) and BeXyl-101060593 (EU-Horizon Europe)

WHAT IS THE RISK OF *XYLELLA FASTIDIOSA* TO ESTABLISH IN TEMPERATE EUROPEAN REGIONS - A BELGIAN CASE STUDY

Casarin N. (1), Hasbroucq S. (2), Pesenti L. (1)*, Géradin A. (1), Emond A. (1), López Mercadal J. (3), Miranda M. (3), Gregoire J. (2), Bragard C. (1)

(1) UCLouvain, Louvain-la-Neuve, BELGIUM; (2) Université Libre de Bruxelles, Bruxelles, BELGIUM; (3) Universitat de les Illes Balears, Palma, SPAIN

**EFSA Young Researchers' Initiative*

To prepare for the potential spread of *Xylella fastidiosa* in northern Europe, addressing data gaps on host plants and insect vectors is crucial. Although the threat to northern temperate areas is considered limited based on climate-suitability modeling, it should not be underestimated considering the impact of *X. fastidiosa* in America. The risk in these regions depends on efficient combinations between host plants and insect vectors. Salicaceae are potential alternate host for *Xylella fastidiosa* and potential insect vectors.

Therefore, *Populus tremula* and *Salix alba,* which are associated with potential insect and commonly found in riparian areas have been investigated. These species are also widely distributed in forests, along roads, and the hydrographic network, potentially acting as stepping stones for the pathogen's establishment and spread. Also, a pilot sentinel plantation was established in the infected region of Majorca to enhance knowledge for pest risk assessment. *Prunus domestica* cv. Opal, *Quercus petraea* and *Salix alba*, along with a network of rosemary 'spy plants,' were used for symptomatic and molecular detection over four years, stressing the need for long-term investigations. Additionally, the flight capacity of *Philaenus spumarius* and *Aphrophora salicina*, potential insect vectors, was studied through mark-release-recapture and flight mill experiments, revealing a possible association with riparian areas and long-distance spread. The results highlight the potential for *Xylella fastidiosa* establishment and spread.

SESSION 2 – Latest findings on *Xylella fastidiosa* resistance and control

HOW MANY PLANT SPECIES CAN *XYLELLA FASTIDIOSA* INFECT? AN UPDATE ON THE EFSA HOST PLANT DATABASE

Delbianco A. (1), Gibin D. (1), Pasinato L. (1), Stancanelli G. (1)

(1) European Food Safety Authority (EFSA)

Following the *X. fastidiosa* oubreak in Italy in 2013, EFSA was requested by the European Commission to provide scientific assistance on this plant pathogenic bacterium. EFSA conducted a pest risk assessment and published several scientific opinions on this topic, that are all included in the EFSA Journal virtual issue on *X. fastidiosa* (https://efsa.onlinelibrary.wiley.com/doi/toc/10.1002/(ISSN)1831-4732.XylellaVI). EFSA also released a database of host plant species of *Xylella* spp.. A systematic literature review allowed the compilation of lists of host plant species, along with collection of information on infection conditions, geographic locations, pathogen taxonomy and tolerant/resistant response of host plants. The database is regularly kept up-to-date with information retrieved through a comprehensive search of the latest scientific literature and EUROPHYT outbreaks notifications by Member States. The raw data are published in Zenodo platform in the EFSA Knowledge Junction community (https://doi.org/10.5281/zenodo.1339343) and interactive reports are available in the freely accessible Microstrategy platform (https://www.efsa.europa.eu/en/microstrategy/xylella). The EFSA database of *Xylella* spp. host plant species represents a key tool for research, risk assessment and risk management. The main findings will be presented, particularly focusing on plant hosts of *Xylella fastidiosa* recently reported.

IDENTIFICATION AND CHARACTERIZATION OF A *XYLELLA FASTIDIOSA* RESISTANT ALMOND GENOTYPE

Vanunu M. (1,3), Zecharia N. (1,3), Shtienberg D. (1), Bar Ya'akov I. (2), Hatib K. (2), Beja R. (2), Holland D. (2), Shemer-Azoulay T. (2), Bahar O. (1)

(1) Department of Plant Pathology and Weed Research, Agricultural Research Organization, Volcani Institute, Rishon Leziyyon, ISRAEL; (2) Unit of Fruit Tree Sciences, Institute of Plant Sciences, Agricultural Research Organization, Newe Ya'ar Research Center, Ramat Yishay, ISRAEL; (3) The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, ISRAEL

Almond leaf scorch (ALS) caused by *Xylella fastidiosa* is an emerging disease in the EU and the Mediterranean that lack effective management tools. To identify *X. fastidiosa* resistance in almond, we grafted seven genotypes on infected almond trees in a commercial orchard, using the commercial cultivar 'Um ElFahem' (UEF) as a susceptible control. Each genotype was grafted on at least 10 trees with 3-4 grafts per tree. Disease incidence and severity were determined visually at 170 days post grafting. Six of the grafted genotypes including UEF, were similarly susceptible to *X. fastidiosa* with 65 to 82% of the grafts showing ALS symptoms. On the other hand, one genotype was clearly more resistant with only 12% symptomatic grafts. In the following year, none of resistant genotype grafts expressed ALS symptoms, while 87% of the UEF grafts were symptomatic. Furthermore, only 12% of the resistant genotype grafts were positive for *X. fastidiosa* in the second year, compared with 50% in the first year. We also measured *X. fastidiosa* migration and relative concentration in the grafts using quantitative-PCR and found that while *X. fastidiosa* migrated to similar distances in UEF and in the resistant genotype, its population was significantly lower in the resistant genotype. This indicates that this genotype is resistant to *X. fastidiosa* and that its resistance intensifies over time. Further characterization of the resistant genotype is underway and will be presented at the meeting.

ENHANCING ROOTSTOCK-MEDIATED SYSTEMIC IMMUNITY AGAINST PIERCE'S DISEASE IN GRAFTED CHARDONNAY

Assis R. (1), Aguero C. (1), Zaini P.A. (1), Dandekar A.M. (1)

(1) Department of Plant Sciences, University of California, Davis, CA, USA.

The introduction of Glassy Winged Sharpshooter (GWSS) in California, a highly efficient vector of *Xylella fastidiosa*, has demanded a more aggressive area-wide chemical mitigation strategy to prevent Pierce's disease (PD) spread in grapes. However, the development of GWSS resistance to pesticides poses a clear and present danger with the potential spread of PD to high value wine growing regions. Our focus is to deploy a genetic solution by using bioengineered rootstocks thatprovide systemic immunity to grafted varietal vines against PD. We demonstrated enhancing systemic immunity to control PD in grapevines grafted to bioengineered rootstocks expressing a secreted chimeric antimicrobial protein (CAP-1). For this study we have bioengineered two commercially relevant and widely used rootstocks, 1103P and 101-14 Mgt, that have been grafted to wild type Chardonnay to evaluate the efficacy of the systemic immunity provided by the bioengineered rootstock. The rootstock has been engineered to express one of seven different CAP proteins to enhance the systemic immunity of the wild type scion and protect it against the development of PD. We plan to compare the efficacy of the six other CAP proteins with the CAP 1 that was validated in the field (Dandekar et al., 2019). All seven versions of CAP are being evaluated in the field, testing their ability to suppress PD in grafted wine grape wild type Chardonnay. The current inventory of 450 grapevines correspond to 69 independent lines. Cane inoculations with *Xf* were performed in the summer of 2021 and spring of 2022. Characteristic symptoms of PD were visible 12 weeks after inoculation and a 0-5 disease index based on number of scorched leaves and the presence of matchsticks was used to score them. We also measured single cordon yields for each vine. Although yield variability was high, probably because plants are relatively young, East (inoculated) cordons displayed yield levels similar to West (non-inoculated) cordons. About 5 to 10 ml of bleeding sap collected from each vine early spring will be analyzed to detect the CAP protein in xylem sap as well as better understand the plant responses to it and to *Xf* virulence factors. The extensive testing, including pathogen challenge, evaluation of plant health, disease development and examination of systemic immunity mechanisms, may lead to the identification of superior bioengineered rootstocks for commercial development considerations.

NOVEL TARGET-ORIENTED PEPTIDES WITH POTENTIAL FOR INFECTION CONTROL STRATEGIES AGAINST *XYLELLA FASTIDIOSA*

Moll dos Santos L.A. (1), Badosa E. (1), Feliu L. (2), Montesinos L. (1), Planas M. (2), Montesinos E. (1), Bonaterra A. (1)

(1) Plant Pathology-CIDSAV, Institute of Food and Agricultural Technology, University of Girona, Girona, SPAIN; (2) LIPPSO, Department of Chemistry, University of Girona, Girona, SPAIN

Peptides can be promising candidates against *X. fastidiosa* due to their broad spectrum of activity and low environmental impact. They can be targeted towards the pathogen by affecting its viability or by hampering processes such as biofilm formation or motility. Peptides can also be directed towards the host by acting as plant defense elicitors, which would induce a priming state. In our studies, a screening platform was developed to assess the bactericidal and antibiofilm activity of peptides using viability qPCR and crystal violet, respectively. Peptides were selected or newly designed, synthetized and tested, resulting in the identification of the peptides 1036 and RIJK2**,** which possess dual activity (high bactericidal and antibiofilm activities). In addition, a screening platform for the identification of plant defense elicitors on *Prunus dulcis*, host of *X. fastidiosa*, was set up with the peptide flg22 using RNAseq and RT-qPCR. From the peptide library studied, we identified the peptide FV7 as a novel plant defense elicitor. In addition to the bifunctional peptide BP178 with bactericidal and plant defense elicitor activities, several of the above new peptides are currently being tested in the almond platform to evaluate their efficacy of infection control against *X. fastidiosa.*

Funding was provided by 101060593 EU project, and RTI2018-099410-B-C21 MCIN/AEI/FEDER, and TED2021-130110B-C43 MCIN/AEI/ Unión Europea NextGenerationEU/PRTR and MCIN FPU19/01434.

GRAPEVINES PRIMED WITH LIPOPOLYSACCHARIDE DEMONSTRATES SYSTEMIC RESISTANCE TO PIERCE'S DISEASE AND REVEALS AN IMPORTANT PEROXIDASE MECHANISM LINKED TO THE IMMUNE MEMORY

Castro C. (1), Melanie M. (2), Her N. (1)*, Disalvo B. (1), Jablonska B. (1), Jeske D. (1), Cantu D. (2), Roper C. (1)

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**EFSA Young Researchers' Initiative*

Plants activate an enhanced defense response when challenged by a pathogen in a mechanism called priming. Molecular signatures of microorganisms known as microbe-associated molecular patterns (MAMPs) can activate priming. The bacterium, *Xylella fastidiosa* (*Xf*), is a xylem-limited pathogen containing lipopolysaccharides (LPS) considered as a MAMP. LPS isolated from *Xf* elicits a priming response from *Vitis vinifera* grapevines. Primed grapevines experienced lower pathogen titer and significantly less disease compared to naïve vines.

During the priming phase (PP) and post-pathogen challenge phase (PPCP), analysis of differential gene expression revealed considerable transcriptional reprogramming. Unlike naïve vines during the PPCP, primed vines revealed an increased number of differentially expressed genes temporally and spatially.

We identified that *VviCP1*, which is a cationic peroxidase, was upregulated during the PP and PPCP. This suggests that *VviCP1* may link the PP to the PPCP. Constitutive expression of *VviCP1* demonstrated significant disease resistance suggesting that a mechanism relying on a peroxidase mediator is important during defense priming to the immune memory. Compared to naïve vines, weighted gene co-expression analysis revealed that more genes were co-expressed in primed vines of local and systemic petioles. This signifies that in primed plants there exists an innate synchronization of the systemic response to combat *Xf*.

HIGH PREVALENCE OF RESISTANT GENOTYPES TO *XYLELLA FASTIDIOSA* IN NATURAL OLIVE RESOURCES DERIVED FROM THE CULTIVAR LECCINO

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In olives, resistance phenomena to highly pathogenic isolates of *Xylella fastidiosa* subsp. *pauca* appear so far to be limited to two cultivars: Leccino (LC) and FS17. In both cultivars mild symptoms, low level of bacterial population and limited distribution in the canopy are consistently detected. Search of resistant cultivars in the Salento peninsula under high inoculum pressure has been exploited for a rapid identification of additional resistant sources, extending the selection to spontaneous olive trees. Since 2016, more than 190 spontaneous olive genotypes, mostly in the productive stage, have been subjected to multiple diagnostic tests, inspections, molecular profiling and paternity test. For 130 of them both parents were identified, of which almost 45% had as candidate parent the cultivar LC, crossed with Cellina di Nardò (CN) or Ogliarola salentina (OG), and in few cases with Nociara and Cipressino. Analysis of the phenotypes throughout the years showed that 70% of these LC-derived genotypes showed traits of resistance or tolerance. Indeed, among the crosses LC x CN only few genotypes turned to be susceptible (9%), while these were predominant in the crosses LC x OG (55%). The resistant phenotype of 3 of these genotypes was confirmed by artificial inoculations while the plant defense response was described by transcriptome analysis. Evaluation of their agronomic traits is undergoing to fully characterize these candidate new cultivars. This work was developed in the framework of the project RESIXO “Strategie per il contenimento del disseccamento rapido dell'olivo: ricerca e studio di Germoplasma Resistente per la Salvaguardia del Patrimonio Olivicolo Salentino" funded by the Apulia Region.

SESSION 3 - *Xylella fastidiosa* vectors biology

REPRODUCTIVE BIOLOGY AND EGG PARASITOIDS OF *PHILAENUS SPUMARIUS*

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The control of *Philaenus spumarius* (Hemiptera: Aphrophoridae), the main vector of *Xylella fastidiosa*, currently relies on measures against nymphs and adults. We studied the reproductive behavior of this insect and its egg parasitoids with the aim of providing new targets and approaches for control strategies against the vector. Main investigations intended to describe: i) a molecular marker of the reproductive phase in females, ii) the duration of ovarian parapause and timing of egg maturation in different climatic areas, iii) the variations in association with the symbiotic bacterium *Wolbachia*, iv) egg parasitoid communities. The results showed that: i) the expression level of the vitellogenin gene is a good marker of egg development phase, ii) the maturation of eggs and the duration of the ovarian parapause is highly dependent on the environmental conditions, iii) the prevalence of *Wolbachia* is highly variable among populations and seems to follow an increasing trend toward colder areas, iv) egg parasitoids [*Ooctonus* spp. (Hymenoptera: Mymaridae)] are present in both North and South Italy. These results start shedding light on some poorly known biological aspects of *P. spumarius*, thus providing useful information for designing new and more effective control strategies that take into consideration the reproductive biology of the main vector of *X. fastidiosa*.

FACTORS DRIVING INSECT VECTOR PRESENCE, ABUNDANCE AND PATHOGEN TRANSMISSION: THE CASE OF *PHILAENUS SPUMARIUS* AND *NEOPHILAENUS CAMPESTRIS*

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Global trade routes have increased the risk of new disease outbreaks and enhance the invasive species spreading worldwide. *Xylella fastidiosa* is one of the vector-borne plant pathogens that provokes huge economic impact. To understand its epidemiology, it is crucial to unveil the ecological factors affecting its insect vectors. *Philaenus spumarius* and *Neophilaenus campestris* are the most important vectors in the Balearic Islands. To shed light on the ecological drivers that influence vector seasonality, a three-year macrocosm study was conducted in olive, vineyard and almond crops in Majorca. Also, transmission tests were conducted under laboratory conditions. We used Generalized Linear Models (GLMs) to assess the effect of different ecological factors (e.g., type of crop or vegetation structure) on the presence and abundance of the vectors. Moreover, we evaluate the differences in transmission of *X. fastidiosa* between both vector species using Generalized Mixed Linear Mixed Models (GLMMs) and Pearson correlation. Our results highlight that the most influential variables explaining the presence and abundance of *P. spumarius* were the vicinity of canopy and cover and for *N. campestris* cover and border vegetation compartments. On the other hand, results on transmission trials showed no correlation between the number of positive insects and the number of plants infected, and that percentage of plants infected did not depend on the species of vector.

ECOLOGICAL ASPECTS OF SPITTLEBUGS OF THE GENUS CLASTOPTERA (HEMIPTERA: CERCOPOIDEA: CLASTOPTERIDADE), POSSIBLE VECTORS OF *XYLELLA FASTIDIOSA* IN OLIVES IN SOUTHEASTERN BRAZIL

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The species of the genus *Clastoptera* are xylem-feeder, can be potential vectors of *Xylella fastidiosa*. In olive orchards in southeastern Brazil, individuals of an as yet unidentified species of *Clastoptera* were frequently observed in association with olive plants. Then, surveys of regions and times of occurrence of nymphs and adults, natural infectivity by *X. fastidiosa*, and association with host plants of *Clastoptera* were carried out to understand their potential involvement in the dissemination of *X. fastidiosa* in olive orchards. Surveys with yellow sticky cards and visual observations in different locations in southeastern Brazil showed that *Clastoptera* occurs only in olive orchards above 1000 m altitude in the Mantiqueira Mountain Range, with population peaks of adults and nymphs between February and April. *Clastoptera* adults collected in cards were positive for *X. fastidiosa* by qPCR, indicating that individuals are naturally carrying the bacterium in olive orchards. By visual observation, adults and nymphs of *Clastoptera* were detected in shoots of olive trees and other shrub or tree plants close to olive orchards, such as *Duranta erecta*, *Salvia rosmarinus*, *Rhododendron* sp., *Tibouchina mutabilis*, and *Prunus persica*. New studies are underway to identify or describe the species of Clastoptera (apparently a new species) collected from olive trees and alternative host plants, as well as to assess their ability to transmit X. fastidiosa to olive trees.

ABILITY OF GLASSY-WINGED SHARPSHOOTER TO ACQUIRE *XYLELLA FASTIDIOSA* SUBSP. *PAUCA* FROM RIPE OLIVE VARIETIES GROWN IN CALIFORNIA, USA.

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Xylella fastidiosa subsp. *Pauca* (*Xfp*) is not currently present in North America but could have significant impacts on the United States olive industry if it were introduced. The state of California (CA), which has historically dealt with disease outbreaks caused by *X. fastidiosa* in other crops, produces 70-80% of olives grown in the US. Several insect species present in CA, including the glassy-winged sharpshooter (*Homalodisca vitripennis*, GWSS) are known vectors of *X. fastidiosa* in grapevine, and could drive spread of *X. fastidiosa* in olive if *Xfp* strains were introduced. This study evaluated the susceptibility of three CA ripe olive varieties (Mission, Manzanillo, and Sevillano) to olive-pathogenic *X. fastidiosa* strain DeDonno, as well as ability of GWSS to acquire this pathogen from infected olive plants. GWSS caged on *X. fastidiosa*-infected olive seedlings for 3 days tested positive by PCR for *X. fastidiosa* at a rate of 4.8%. This shows that GWSS can acquire *Xfp* from CA ripe olive varieties and could potentially act as a vector of *X. Fastidiosa* in this crop. Although overall acquisition rates were low, acquisition occurred as soon as 30 days post-inoculation of the plants in all three olive cultivars tested. Potential for GWSS to serve as a vector of *Xfp* in olive is relevant to scenarios where this subspecies is introduced to North America, or where GWSS is introduced to areas where *Xfp* is already present such as the Mediterranean.

DEGREE-DAY-BASED MODEL TO PREDICT EGG HATCHING OF *PHILAENUS SPUMARIUS* (HEMIPTERA: APHROPHORIDAE), THE MAIN VECTOR OF *XYLELLA FASTIDIOSA* IN EUROPE

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Philaenus spumarius L., the main vector of *Xylella fastidiosa* (Wells) in Europe, is a univoltine species that overwinters in the egg stage, and its nymphs emerge in late winter or spring. Predicting the time of egg hatching is essential for determining the precise times for deploying control strategies against insect pests. Here, we monitored *P. spumarius* eggs from oviposition to egg hatching together with the daily temperatures and relative humidities at four field locations located at different altitudes in central Spain. The collected data were used to build a growing degree day (GDD) model to forecast egg hatching in the Iberian Peninsula. Furthermore, the model was validated with field observations on the presence of newly born nymphs in different regions of Spain. The model was then used as a decision-support tool to calculate the optimum timing for applying control actions against *P. spumarius* nymphs. Our results suggest that controlling nymphs at two different dates would target the highest percentage of nymphal populations present in the field. Our model represents a first step for predicting the emergence of nymphs and adopting timely control actions against *P. spumarius*. These actions could limit disease spread in areas where *X. fastidiosa* is present.

VECTORS AS SENTINELS OF PLANT DISEASES IN A CHANGING WORLD: RISING TEMPERATURES INCREASE THE RISK OF *XYLELLA FASTIDIOSA* OUTBREAKS

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Not much is known about how a changing climate will affect the epidemiology of generalist vector-borne diseases. We developed a high-throughput screening method to test for the presence of *Xylella fastidiosa*, in its insect vectors. Based on a four-year survey in climatically distinct areas of the island of Corsica (France), we found a significant positive correlation between the frequency of vectors positive for the bacterium and temperature. Higher prevalence corresponded with milder winters. Climate projections show that the risk for *X. fastidiosa* outbreak will increase in the future. Besides calling for research efforts to limit the incidence of plant diseases in temperate zones, our work reveals that recent molecular technologies could and should be used for massive screening of pathogens in vectors in order to scale-up surveillance and management efforts. Such methods could for example target multiple plant-pathogens in vectors communities. This will contribute to better understand the drivers of plant pest spread and establishment such as the influence of climate change or ecosystem degradation or to better evaluate environmentally sound control solutions over large geographical and temporal scales.

SESSION 4 – Tools for *Xylella fastidiosa* surveillance and detection

USE OF PLASMID PROFILES IN EPIDEMIOLOGICAL SURVEILLANCE OF *XYLELLA FASTIDIOSA* OUTBREAK IN THE VALENCIAN COMMUNITY, SPAIN

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**EFSA Young Researchers' Initiative*

In the *Xf* Demarcated Area (DA) of the Valencian Community (VC), Spain, all infected plants and insect vectors harbor the same sequence type (ST) as identified by MLST analysis (*Xf* subsp. *multiplex* ST6). However, *Xf* strains harboring three plasmid profiles have been isolated to date in this DA: strains harboring one (pUCLA-ESVL) or two (pXF64-Hb_ESVL and pUCLA-ESVL) plasmids, or no plasmids. In this study, we used a PCR-based plasmid-typing approach as a tool in the epidemiological surveillance of this *Xf* outbreak. A set of hundreds of DNA samples testing positive by qPCR including infected almond trees (86% of samples), 20 plants species including crops and landscape plants identified as *Xf* hosts in this DA, as well as three species of *Xf* insect vectors (3% of samples) were typed for the presence of the two plasmids. Results revealed that four plasmid profiles were present in this DA, with a new plasmid profile being identified, that did not match any strain isolated to date. The plasmid profile most abundant was pXF64-Hb_ESVL(-)/pUCLA-ESV(-) (55%), followed by pXF64-Hb_ESVL(+)/pUCLA-ESVL(+) (24%); however, its distribution differed among municipalities being some of them more prevalent in specific regions. The geospatial associations of the different plasmid profiles and the implications in the surveillance of the *Xf* epidemic in the DA of the VC will be discussed.

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EARLY DETECTION OF *XYLELLA FASTIDIOSA* INFECTION USING REFLECTANCE PROXIMAL SENSORS IN ALMOND AND OLIVE TREES

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**EFSA Young Researchers' Initiative*

Asymptomatic infection, long incubation period and non-specific symptoms hamper early detection of *Xylella fastidiosa* (*Xf*), which is essential to successfully eradicate, contain and manage *Xf* epidemics. Our objective was to assess the response of *Xf*-infected plants by physiological traits using proximal sensors at leaf level in (i) artificially infected olive plants and (ii) naturally infected almond trees in Mallorca Island. Olive leaves were measured, sampled and tested for *Xf*-infection for 18 months. Almond *Xf* symptoms were evaluated in 150 trees in May, June and July 2022. One branch was selected per tree and leaves measured and tested each month. Our results identified, in both species, physiological traits related to anthocyanins, carotenoids, xanthophylls content as the best indicators to discriminate between plants *Xf*-infected (even in absence of visible symptoms) from those non-inoculated or testing *Xf*-negative. Hierarchical clustering and discriminant analyses showed distinct groups differentiating symptomatic from asymptomatic leaves. Moreover, machine learning algorithms revealed the predictive capability of the visible and infrared spectral information to detect *Xf* infection in asymptomatic almond leaves 1 or 2 months before symptoms appearance with overall accuracies >87%. These results allow the application of imaging techniques, which is being used in a phenotyping platform.

Funding: XF-ACTORS, ITS2017-095(CAIB), E-RTA2017-00004-C06-02 (AEI-INIA), OIAOE, BeXyl

DETECTION OF SYMPTOMS INDUCED BY *XYLELLA FASTIDIOSA* WITH HIGH-RESOLUTION MULTISPECTRAL SATELLITE DATA: ASSESSMENT WITH AIRBORNE HYPERSPECTRAL IMAGERY

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Xylella fastidiosa (Xf) and *Verticillium dahliae* (Vd) are plant vascular pathogens that threaten global olive and almond production, restricting the flow of water and nutrients. Previous studies have demonstrated that hyperspectral and thermal imagery acquired at high spatial resolution successfully detected early symptoms of infection. Here, we assessed the feasibility of scaling-up Xf- and Vd-detection algorithms to high-resolution multispectral satellites Worldview-2 and -3 (WV 2&3) against airborne hyperspectral imagery in olive and almond orchards acquired across countries and seasons. Machine learning algorithms were trained using as inputs model-inverted plant traits, solar-induced fluorescence (SIF), and a thermal indicator (CWSI). Results obtained with multispectral satellite and airborne hyperspectral datasets reached similar accuracies for detecting advanced stages of disease development (OA=0.81/0.91; k =0.63/0.81, respectively). However, the detection of the early stages of disease development was outperformed by hyperspectral data (OA=0.74 and k=0.47 for WV 2&3) due to the inability of WV 2&3 to track critical plant traits such as NPQI, xanthophyll proxy PRIn, SIF, or anthocyanin content. Adding the thermal CWSI indicator to satellite data improved OA by 10%–15%, but the early detection of symptoms still requires specific narrow bands sensitive to Xf- and Vd-induced symptoms. Comparisons across species (almond, olive) and pathogens (Xf, Vd) will be discussed.

RIPEST: EFSA DEVELOPS A TOOL FOR DESIGNING MORE ROBUST AND HARMONIZED PEST SURVEYS IN THE EU

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Since 2017, on requests of the European Commission, EFSA provides support to the EU Member States in the planning and execution of plant pest surveys. In this context EFSA developed a methodological framework and a related toolkit for surveying Union quarantine pests. The EFSA toolkit for pest surveys supports the Member States in the preparation, design and reporting of statistically sound and risk-based surveys. In addition, EFSA is further developing tools and methods to assist Member States in optimizing their survey efforts.

In this presentation, the conference participants will be guided through the preparation, design and reporting of statistically sound detection surveys for *Xylella fastidiosa* in the EU Member States. The authors will focus on the setting of the different survey parameters that derive from the characterization of the bacterium, of its host plants and of the methods of detection and identification. The toolkit clearly contributes to more robust and harmonized surveillance system in the Member States, allowing for comparison of surveys designed and conducted for different areas and in different years.

DELIMITING SURVEY OF *XYLELLA FASTIDOSA* SUBSP. *MULTIPLEX*: BRINGING TOGETHER MULTIDISCIPLINARY EXPERTISE TO SUPPORT RISK MANAGEMENT

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Efficient delimitation of a newly detected area infested by a quarantine pest is a key element to enhance the effectiveness of phytosanitary measures. *Xylella fastidiosa* subsp. *multiplex* was first detected in the department Aude (Occitanie, France) in September 2020. In the following months, the reinforced surveillance implemented in accordance with Regulation (EU) 2020/1201 resulted in numerous detections close to the first outbreak, while an extra survey plan made it possible to detect several new and separate infested areas in the same department. Then, in 2021 a multidisciplinary working group in the framework of the Epidemiological Plant Health Surveillance Platform was appointed to support the risk manager with the further delimitation of the contaminated area. On-site observations, expertise and risk maps using eco-climatic data were used to define a risk-based and sequential survey plan and provide guidelines to improve the surveillance. The implementation of this survey plan resulted in findings of both contaminated and non-contaminated areas. The risk manager strongly relied on the results of this survey plan to adapt the surveillance and management strategy. This approach could be generalised to other outbreaks of *Xylella fastidiosa* or any other pest. It highlights the benefits of bringing together researchers (bacteriologists, entomologists, epidemiologists), inspectors, agronomists and stakeholders to devise a delimiting survey.

EVALUATION OF OUTBREAK RESPONSE PLANS FOR *XYLELLA FASTIDIOSA* IN ALICANTE, SPAIN

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Xylella fastidiosa is a priority quarantine pathogen in the EU. As laid down in the EU legislation, after an outbreak, a demarcated area is established delimiting the infested zone surrounded by a buffer zone, where intensive surveillance and control measures are applied. The spread of almond leaf scorch disease (ALSD) in Alicante, Spain, was simulated with a spatial individual-based epidemiological model, comparing the performance of different surveillance and control designs for outbreak management. Two survey designs were compared where the survey effort was estimated based on the population size, or in two-steps, first the sample size per hectare, and second the hectares to be inspected. Two confidence levels, buffer zone sizes and eradication radii were compared, including those set by the regulation. The effect of vector control and inoculum removal was considered in the model by reducing the transmission rate parameter in the buffer zone, greatly decreasing the number of infected trees. Without the reduced transmission rate, the number of infected trees was similar to that obtained without any intervention, even with the eradication measures in place. No major effects were observed with the different buffer zone sizes and eradication radii. Higher confidence levels resulted in better disease control but increased survey efforts. The two-step approach had the greatest survey efforts but, did not improve disease control substantially.

Posters session

Sunday 20 August, 12.40-13.30: presentation of posters with odd numbers

Sunday 20 August, 13.30-14.20: presentation of posters with even numbers

1. CURRENT SITUATION OF *XYLELLA FASTIDIOSA* IN FRANCE

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Xylella fastidiosa (*X. f.*) has been detected for the first time in France, in Corsica and PACA region during the summer 2015. Since 2020, *X. f.* has been also highlighted in Occitanie region with a significant number of new outbreaks discovered during the summer 2022. Only the subspecies *multiplex* has been reported so far in these regions, although *X. f.* subsp. *pauca* was previously reported and eradicated in one outbreak in Menton (PACA). In France, the sequence-types (ST) ST6 and ST7 were identified, but new ST88 (Var) and ST89 (Alpes-Maritimes) were recently identified in PACA on two different areas with a limited host range. In Occitanie, only ST6 has been identified but MLVA analyses suggested theses strains are genetically different from ST6 strains from PACA, Corsica and Spain. Since 2015 in France, only 5 olive trees (*Olea europaea*) have been found infected with low titer. No grapevine (*Vitis vinifera*) and no *Citrus* sp. were found infected with *X. f.*.
Official *X. f*. detection is based on the real-time PCR Harper *et al.*, 2010. The new version of the French method MA039 v6 includes an internal PCR control 18S (Ioos *et al*., 2009) for the detection in plant and a new DNA extraction method based on the Maxwell® HT Environmental TNA kit (Promega). Compared to the traditional CTAB-Chloroform method, this new automatized protocol allows an improved sensitivity on *Olea europaea* and *Quercus* spp., safer working conditions for operators and the automatization of the process.

2. AUSTRALIA PREPAREDNESS: *XYLELLA FASTIDIOSA* AND ITS INSECT VECTORS – POTENTIAL CONTROL AND MANAGEMENT OPTIONS APPROPRIATE TO AN AUSTRALIAN CONTEXt

Lesieur V. (1,2), Kaye L. (2), Caron V. (3), Rafter M. (2), Lee J. (4), Djordjevic-Ilic M. (4), Mcwhirter I. (4), Hunter G. (3)

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The spread of *Xylella fastidiosa* (Xf), one of the most detrimental plant pathogens currently affecting the Americas and Europe, is a reality that will likely continue due to globalisation. As such, effective preparedness of countries where the pathogen does not yet occur is paramount to shield both native flora and commercial plant-based industries from an incursion and spread. As a first step for being prepared for a robust response should an incursion take place, a comprehensive and critical review of the existing literature regarding available control and management strategies was undertaken.

Here we will present the results of this review which was a response to the Australian National Xylella Action Plan and specifically Action 3.4 which is to Analyse literature and overseas experience to identify control and management options relevant to the Australian context.

Our results indicated that current mitigation strategies are largely deployed across those that target Xf in the host plant and those which focus on the insect vectors with the aim to minimise the impact of Xf, its vectors and the associated plant diseases caused by the bacterium. Furthermore, integrating these results, we have identified several research gaps that could limit Australia's capacity to respond to a Xf incursion. We have therefore proposed a set of 24 research recommendations that will increase Australia's preparedness to control and manage a Xf outbreak and these recommendations will be discussed.

3. SURVEY ON THE PRESENCE OF *XYLELLA FASTIDIOSA* IN OAK TREES IN FRANCE AND ASSESSMENT OF SEED-TRANSMISSION

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Xylella fastidiosa (*Xf*) is a plant xylem-inhabiting bacterial pathogen that may colonize a wide range of plants and cause diseases in various crops of economic importance. According to climate model predictions, most French area could be suitable to *X. fastidiosa* subsp. *multiplex* (*Xfm*). Strains of this subspecies are likely to cause serious damages to various plant species including forest and shade tree species such as oak. A sampling campaign was therefore conducted in 2020 and 2021 in French state forests exploited for the quality of their oak trees and in woods. Quantitative PCR was used to detect *Xf*. None of the samples taken from oak trees during these two years revealed any *Xf* contamination. Whereas *Xf* introduction through contaminated plant material (horizontal transmission) most probably already occurred into Europe, *Xf* vertical transmission through seed is poorly documented. The transmission of *Xf* to the acorn was studied by inoculating stems at different vegetative stages (from flower to fruit). Transmission from acorn to seedling was also analysed by acorn inoculation before sowing. In both types of analyses, no *Xf* transmission was demonstrated, while stem colonization was observed. However, the question of seed transmission for other plant species remains an important issue for a better understanding of the mechanisms underlying the epidemiological dynamics and control of diseases caused by this European priority pathogen.

4. DIRECT *XYLELLA FASTIDIOSA* WHOLE GENOME SEQUENCING FROM PLANT USING TARGETED ENRICHMENT

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Xylella fastidiosa is a gram-negative plant pathogenic bacterium with a broad host range, which causes important diseases. Endemic to the Americas, this bacterium was recently introduced to the European continent where it has been detected since 2013 in various plant species in Italy, France, Spain, and Portugal. Precise genetic characterization of *X. fastidiosa* diversity can be obtained using whole genome sequencing, but isolation of the bacterium from contaminated plant material is not always successful. In order to access the bacterial genome directly from plant infected samples, a SureSelect targeted enrichment method was developed. Enrichment was very efficient to retrieve *X. fastidiosa* genome sequence with genome coverage significantly improved for all enriched plant samples, whatever the plant species or the contamination level. Whole genome sequencing data are useful to study the epidemiology and the origin of *X. faxtidiosa* introductions, and to give new insights for surveillance and management of this quarantine bacterium.

5. EVALUATION OF A NEW BIOLOGICAL SOLUTION COMBINING ANTIMICROBIAL PEPTIDES AND A DELIVERY SYSTEM BASED ON *AGROBACTERIUM TUMEFACIENS* FOR THE CONTROL OF *XYLELLA FASTIDIOSA*

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Xylella fastidiosa (Xf) is a quarantine bacterium that can infect the xylem of a wide range of cultivated, ornamental, and wild host plants. Xf is the causal agent of Pierce's disease in grapevine in North America, citrus variegated chlorosis in South America, and olive quick decline syndrome, notably in Southern Italy. There is currently no method that effectively prevents or cures host plants from infection. The current approaches for Xf disease management mainly rely on eradication of infected plant to reduce inoculum source, the use of insecticides to control the vector populations, and the use of healthy plant propagation material. Therefore, there is a need for new and safe biological solutions. Protecting and/or curing mature fruit trees infected by *Xf* remains a challenge but would be the best possible solution to avoid eradicating infected trees and replanting. The use of antimicrobial peptides (AMPs) represents an interesting approach due to their broad spectrum of activity against bacterial pathogens, low environmental impact, and limited evolution of resistance. EBCL will test an innovative approach combining the use of AMPs and a delivery system based on Agrobacterium tumefaciens. The main advantage of this method is that it can potentially confer resistance to mature fruit trees. Preliminary tests will thus be performed to evaluate proof of concept of this strategy for the control of Xf.

6. *XYLELLA FASTIDIOSA*: ACTION OF THE ENZYME LESA ON PLANT LIPIDS

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Xylella fastidiosa is classified as a global threat for agriculture. After inoculation by xylem sap-feeding insects, *Xylella fastidosa* colonizes plants by different strategies including an enzymatic arsenal. Among these molecules, LesA[1] is the major enzyme among the proteins secreted by the bacteria. According to its sequence and its structure, this protein is supposed to be a member of lipase/esterase family. LesA is structurally close to LipA of *Xanthomonas oryzae* that is also involved in virulence. We produced LesA in *E. coli* and performed a number of experiments of characterization by in-vitro tests based on selected substrates to verify its activity. In a second step, we performed an untargeted lipidomic approach using total lipid extracts from *Arabidospis thaliana* leaves. The results obtained on the activity tests confirm the esterase activity of LesA but only on short carbon chains. The lipidomic results show 172 compounds significatively differents (p<0,05) between treated and untreated samples. Some have been identified by mass-spectrometry, suggesting that LesA could have an impact on some phospholipids. More investigations are needed to better know biological substrates of LesA and its role in plant colonization and symptoms expression in plants infected by *Xylella fastidiosa*.

[1] Nascimento, R. et al. The Type II Secreted Lipase/Esterase LesA is a Key Virulence Factor Required for *Xylella fastidiosa* Pathogenesis in Grapevines. Sci. Rep. 6, 18598 (2016)

7. THE FATE OF *XYLELLA FASTIDIOSA* IN COMMON WOODY PLANT SPECIES IN BELGIUM

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In 2018, *Xylella fastidiosa* (Xfas) was detected at a Belgian arboriculture wholesaler, in olive plants imported from Spain. Despite this being a sole finding, its capacity to establish under regional environmental conditions was investigated. Colonisation of Xfas was assessed in 20 perennial woody plant species, common in the urban landscape and natural environment, under both greenhouse (10-35°C) and outdoor conditions. In three consecutive years, shoots were pin-prick inoculated with strain CFBP 8431 (ST6) from the subsp. *multiplex*. The fate of the bacterium was monitored by Harper TaqMan PCR and dPCR after 5, 12, 24 and 36 months. Overall, plants maintained a healthy appearance. Xfas detection in the inoculated part of the shoots did not indicate multiplication. Indoors, bacterial titres remained fairly constant over time, whereas they were generally lower and less stable in outdoor plants. There was also no clear evidence of bacterial movement as detections in non-inoculated twig parts were mostly in the grey zone of quantification, except for *Castanea sativa*, *Populus nigra* and *Quercus robur*. Although a slow coloniser, xylem microbiome analyses of *Salix alba* revealed that the presence of Xfas can significantly alter resident endophytic bacterial communities at a local scale. This study shows that under the regional climatic conditions, establishment and proliferation of Xfas is unlikely, yet the pathogen may remain latent in infected tissue for an extended period of time.

8. FLUORESCENCE IN SITU HYBRIDIZATION COUPLED TO FLOW CYTOMETRY AND CELL IMAGING ANALYSES AS POTENTIAL TOOLS FOR *XYLELLA FASTIDIOSA* RESEARCH AND DIAGNOSTICS.

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Fluorescence in situ hybridization (FISH) is used to directly identify microorganisms within complex samples in a few hours having a widespread application in environmental microbiology, including reports for Xylella fastidiosa detection in plant xylem vessels and insect honeydew. In Costa Rica, *X. fastidiosa* subsp. *fastidiosa* is endemic, with high bacterium prevalence and seldom disease manifestations in coffee plantations throughout the central valley, where most of the coffee is grown. We study the extent of the bacterial infection in coffee and its insect vectors. For this purpose, we implemented two FISH-based tools coupled to (i) flow cytometry (Flow-FISH), and (ii) cell imaging analyses, to study specific events of the X. fastidiosa life cycle in vitro and in vivo; as well as for the direct detection of X. fastidiosa with potential diagnostic value. For this purpose, we tested an *X. fastidiosa*-specific probe (KO210) and a universal probe (Eub338), using flow cytometry and microscopy. We standardized the hybridization conditions using an *X. fastidiosa* pure culture, we determined the detection limits of each tool, and confirmed the specificity of the KO210 probe for *X. fastidiosa*. Applications of the tools include detection of bacteria from plant and insect material, and in vitro cultures, with an in-depth qualitative and quantitative resolution, broadening the existing tools for research and diagnostics in *X. fastidiosa*.

9. IMPROVING THE DIAGNOSTIC CAPABILITY FOR *XYLELLA FASTIDIOSA* SUBSP. *MULTIPLEX* USING ADVANCED NANOTECHNOLOGY AND PCR-BASED ASSAYS

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Xylella fastidiosa subsp. *multiplex* (Xfm) is an emerging threat to the southern U.S. pecan and peach growing regions, causing pecan bacterial leaf scorch and phony peach disease, respectively. The current lack of reliable diagnostic tools for Xfm causes uncertainty in the specialty crop industry in the U.S. The research objective is to improve diagnostic capability for Xfm. We validated different diagnostic tools for Xfm using enzyme-linked immunosorbent assay (ELISA) and molecular-based assays, including traditional PCR and real-time quantitative PCR (qPCR). New Xfm-specific primers were generated and validated, producing expected amplicons specific to Xfm. We also developed a TaqMan qPCR assay protocol for the detection of Xfm in pecan and peach. The results of the qPCR experiments based on plant genomic DNA from petioles were equivalent to the traditional PCR amplification based on crude sap. Further improvement in detection methods is critical for the early diagnostics and subsequent timely intervention for Xfm. To address this, advanced nanotechnology was used to improve diagnostic capability. Antibody-conjugated immunomagnetic carbon nanotubes were developed to improve sensitivity and reliability of PCR-based diagnostics for Xfm. The improved PCR diagnostic methods will support production and distribution of clean plant materials during national and international movement of plant materials.

10. DETECTION OF *XYLELLA FASTIDIOSA* BY COLORIMETRIC LAMP AND DROPLET DIGITAL PCR ASSAYS

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Xylella fastidiosa (Xf) is a bacterium that inhabits the xylem vessels of over 600 plants. The entry of Xf subsp. *pauca* ST53, causing the olive quick decline syndrome in Italy, represents the first outbreak in field on UE territory.

Prompt field-based and sensitive laboratory assays are urgently required for the containment strategies of the Xf spreading. A cLAMP and ddPCR assays, based on widely validated primer (Harper *et al.,* 2010), were used for Xf detection in a crude alkaline sap obtained from incubation of thin olive stem slices in NaOH-based buffer. cLAMP assay can be performed at any location in a portable isothermal block at 65°C, providing a naked-eye detectable amplification result in 40-minutes. Both assays detected the target DNA up to 10^2 CFU/mL of bacterium in crude alkaline sap without any inhibition effect. Moreover, a ddPCR assay was implemented to detect Xf in purified DNA extracts prepared from different plant and insect matrices artificially contaminated with Xf suspension..

An exstensive validation of both assays on naturally infected samples, using crude extracts and purified DNA, proved that cLAMP has remarkable potential as first screening test for a timely detection of Xf at the point of care due to its simplicity, low cost, and portability; conversely ddPCR, although requires expensive reagents/equipment and is time-consuming, is more sensitive, especially using purified DNA extracts and solves some results recorded as doubtful by the qPCR assay.

11. XYLEM SAP RECOVERED FROM DIFFERENT CROP SPECIES AFFECTS IN VITRO GROWTH OF *XYLELLA FASTIDIOSA* SUBSP. *PAUCA*, STRAIN DE DONNO

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**EFSA Young Researchers' Initiative*

Mechanisms driving *Xylella fastidiosa* lifestyle and the host susceptibility are still largely unknown. An example of successful host adaptation is given by *X. fastidiosa* subsp. *pauca* (*Xfp*) infecting olives, with a large number of cultivars (cvs) being susceptible, and resistance traits identified only in the olive cvs Leccino and FS17. In this study, the in vitro growth and gene expression of *Xfp* were evaluated in artificial liquid media enriched with xylem sap from: olive cvs Leccino (resistant) and Cellina di Nardò (susceptible), and from citrus and grapes representing *Xfp* immune species. The results show that the sap of Cellina di Nardò promoted a greater planktonic growth than Leccino. Conversely, a higher biofilm formation was detected when the sap of Leccino was used. Media supplemented with citrus sap produced the lowest values, both for biofilm formation and planktonic growth. Unexpectedly, values similar to those recorded for Cellina di Nardò were obtained using the sap from grapes. The level of expression of 15 *Xfp* genes involved in several biological processes was assessed. The multivariate analysis yielded two clusters, one including Cellina di Nardò and grapevine, and the second Leccino and citrus. These results suggest that the xylem sap composition contributes to the resistance/susceptibility of olive cvs, but as showed by the contrasting results gathered with citrus and grapes, most likely this is only one of the components of the multifactor response to *Xfp*.

12. XVETORES.PT: BIOLOGY OF XYLEM-SAP FEEDING INSECT VECTORS AND POTENTIAL VECTORS OF *XYLELLA FASTIDIOSA* IN PORTUGAL

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EFSA Young Researchers' Initiative

Xylella fastidosa was reported for the first time in Portugal in 2019; since then, this phytopathogenic bacteria was progressively spread throughout the country, and currently, 17 demarcated regions of infection have been established. This constant spread may lead to devastating economic and environmental problems, threatening Portugal's agricultural sector and plant diversity. Since there is no cure for this bacterium, vector control is perceived as the main tool to limit the spread of *X. fastidiosa*. In Portugal, some studies developed in the Northeast region demonstrated the importance of the main and potential vectors and clarified aspects of biology, diversity and abundance; however, there is a lack of knowledge about these aspects at the national level. Therefore, the project Xvetores.pt: Biology of xylem-sap feeding insect vectors and potential vectors of *Xylella fastidiosa* in Portugal, funded by EFSA (call GP/EFSA/ALPHA/2021/07), aims to study the biology, abundance, and diversity of insect vectors and potential vectors in different agrosystems such as olive groves, cork oak forests, and urban and semi-natural areas in different regions of Portugal with different agroclimatic conditions. Furthermore, preference studies for host plants and studies on the biology of vectors under microcosm conditions will also be carried out. The Xvectors.pt project is led by IPB and integrates five other Portuguese partners, namely ISA/ULisboa, INIAV, DGAV, UP, and InPP.

13. MEDITERRANEAN AGRICULTURAL SPECIES, THEIR VOLATILES, AND THE POTENTIAL IMPACT ON *PHILAENUS SPUMARIUS*

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Philaenus spumarius (Linnaeus, 1758) (Hemiptera, Aphrophoridae) is Europe's most common and widespread xylem-sap feeder insect. This insect has become a serious threat to European agriculture due to its recognized role in transmitting the phytopathogenic bacterium *Xylella fastidiosa* (Xanthomonadales: Xanthomonadaceae). Therefore, understanding the factors that influence the host plants' choice by this insect vector could be an important tool to manipulate the vector behaviour and implement sustainable control strategies. Therefore, in this work, the volatile profile of the leaves of three important Mediterranean crops, almond, olive, and vine were assessed. Moreover, the P. spumarius olfactory response towards two of the most abundant and common Volatile Organic Compounds (VOCs) (cis-3-hexenyl acetate and cis-3-hexen-1-ol) present in the crop leaves were evaluated. From the three plant species, in total, 83 compounds were identified. Although the plant species showed a distinct volatile profile, cis-3-hexenyl acetate and cis-3-hexen-1-ol were common and abundant. Furthermore, females of P. spumarius were significantly attracted to cis-3-hexenyl acetate and cis-3-hexen-1-ol, whereas males did not respond to the VOCs. Our results can help future implementation of approaches to manage the vector and the spread of *X. fastidiosa*.

14. *XYLELLA FASTIDIOSA*: A EUROPEAN THREAT UNDER CLOSE SURVEILLANCE ON THE MEDISYS PLATFORM

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In December 2016 EFSA was mandated by the European Commission to carry out a horizon scanning exercise on emerging plant pests. From that moment, and in close collaboration with the Joint Research Centre, EFSA started its monitoring and extraction of news and scientific data on plant pests based on the Medisys platform (https://publications.jrc.ec.europa.eu/repository/handle/JRC53155).

Classification is automatically and continuously carried out by the system on more than 21,000 media and scientific literature sources, from around 200 countries, using a set of more than 13,000 pre-defined keywords. The result of this activity is presented in freely available monthly newsletters (https://efsa.onlinelibrary.wiley.com/doi/toc/10.2903/(ISSN)1831-4732.Horizon-scanning-for-plant-health).

With its 54 keywords, the European situation of *Xylella fastidiosa* has been monitored over the last 6 years on media and scientific publications, from outbreaks to vectors, from subspecies to hosts, from detection methods to epidemiology. Since the beginning of this activity, *X. fastidiosa* was the pest providing the largest number of items on Medisys and the most represented pest in the horizon scanning publications (in 94 outputs over the total 108 accessible by March 2023).

15. ENDOPHYTIC MICROBIOMES OF WILD PLANTS SUSCEPTIBLE TO *XYLELLA FASTIDIOSA* INFECTION

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Xylella fastidiosa (Xf) is a phytopathogenic bacterium responsible for a wide range of diseases with high economic, environmental and social impacts. The plant-associated microbiome plays a central role in maintaining fitness, but little is known about the diversity and structure of these endophytic microbial communities. It is therefore essential to study this diversity and the interactions between endophytes and plants in order to understand the biotechnological potential of these microorganisms. In this context, we aim to characterise the structural diversity of endophytic microorganisms in wild plant species susceptible to Xf infection and present in the demarcated area of Portugal. Plant samples were selected and tested by qPCR for the presence of Xf. The microbiome was analysed in plants with and without Xf using a long-read sequencing approach. The results suggest a functional importance of some core microbiome groups, namely related to the ability to degrade toxic substances, fix nitrogen and promote plant growth. They may also help to explain the lack of visible symptoms and signs of decline in these wild plants, suggesting that certain taxonomic endophyte groups may contribute to modelling the infection. Notably, this is the first study in wild plants and the identification of relevant clusters is a contribution to the understanding of the impact of Xf on microbiome dysbiosis in nature.

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16. LONG-READ METABARCODING APPROACH FOR DIAGNOSIS AND EPIDEMIOLOGY IN GENETICALLY HETEROGENEOUS *PRUNUS* SP. ORCHARDS

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Diagnostic challenges are increasing as globalisation and climate change favour the introduction of new plant pests. *Xylella fastidiosa*, *Xanthomonas arboricola*, *Pseudomonas syringae* and *Monilinia fructicola* are important pests affecting Prunus production with significant losses. Two different Prunus production systems were analysed: old orchards of *P. dulcis* and coexisting orchards of *P. avium* and *P. persica*, where significant areas of *P. dulcis* have recently been established. The high density of genetically heterogeneous *Prunus* sp. in which pests can coexist favours the development of highly adapted strains and/or the occurrence of coevolutionary phenomena associated with the spread and emergence of new diseases. This coexistence of phylogenetically close strains with drastically different phenotypes is a critical diagnostic challenge. In this context, molecular epidemiological studies were conducted by characterising the leaf and flower bacteriome and mycobiome of *Prunus* sp. using a long-read sequencing approach to assess the occurrence, distribution and impact of pests. Preliminary results showed that, in contrast to the leaf microbiome among Prunus species, significant differences were found between the flower and leaf microbiomes. Important pathogen-related groups were detected with significant impact on the microbiome structure, supporting the use of this approach for large screening phytosanitary surveys.

This work was financed by FCT (PTDC/ASP-PLA/3145/2021).

17. PHENOLOGY AND ECOLOGY OF *PHILAENUS SPUMARIUS* L. INFORM RISK ASSESSMENT FOR *XYLELLA FASTIDIOSA* WELLS IN SWITZERLAND

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The meadow spittlebug *Philaenus spumarius* L. (Hemiptera: Aphrophoridae) has been identified as the main vector of *Xylella fastidiosa* Wells in Europe. With a proactive approach toward a potential arrival of *X. fastidiosa* in Switzerland, we are studying the phenology and ecology of *P. spumarius* in the southern Alps so as to inform risk assessment for *X. fastidiosa* and suggest timely and effective management measures. The study of phenology allowed to identify the developmental periods, the host plants and their specificity for the nymphs in the Insubric climate. The ecology was studied across Canton Ticino according to a stratified design that considered different combinations of environmental factors (climate, geology and topography, summarized by the Swiss Environmental Domains), habitat (vineyards, olive groves, orchards, hay meadows and pastures) and management (intensive vs. extensive). This provided information about host plants preference and specificity within different regional pools, and estimates of the density of *P. spumarius* in different habitats. Nymphs select preferentially host plants from Asteraceae, Plantaginaceae, Caryophyllaceae, Fabaceae families, in particular *Taraxacum officinale*, *Plantago lanceolata*, *Silene vulgaris*, *Trifolium pratense*. Nymphs' host preference varies according to regional pools and habitat type. Density of spittle nests is significantly higher in hay meadows compared to the other surveyed habitats and in extensively-managed habitats.

18. MOBILE DIGITAL DATA COLLECTION TOOLS: *XYLELLA FASTIDIOSA* OUTBREAK PREPAREDNESS & PLANT HEALTH SURVEILLANCE

Lines A. (1)

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Xylella fastidiosa poses a significant threat to UK plants, with the potential to cause widespread damage. As infected hosts may have long asymptomatic periods, it is crucial to have effective tools for early detection and rapid response to Xylella outbreaks. We developed a digital data collection system to coordinate Plant Health Inspectors during a rapid response to Xylella outbreaks. The system uses ESRI ArcGIS Online applications to generate survey grids around positive findings and allocate grid squares directly to inspectors. Barcodes attached to samples and scanned into the ESRI Field Maps mobile app provide full traceability and controlled data entry ensures consistency. All collected data is available on ESRI Dashboards for immediate analysis, ensuring that policy decisions and data analysis are always based on the latest data. Full deployment and training for 400+ inspectors were completed in 10 months. The system has proved to be effective in directing sample collection and providing real-time information to management teams during simulated outbreaks. These digital tools are essential for mitigating the impact of Xylella on UK agriculture and horticulture.

19. GENOMIC CHARACTERIZATION OF *XYLELLA FASTIDIOSA* SUBSP. *PAUCA* STRAINS ISOLATED FROM OLIVE (*OLEA EUROPAEA* L.) AND ALMOND (*PRUNUS DULCIS* MILL.) TREES IN ARGENTINA

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EFSA Young Researchers' Initiative

In Argentina, *X. fastidiosa* (Xf) subsp. pauca is considered a regulated non-quarantine pest that is causing serious phytosanitary problems in traditional olive plantations over 50 years old, especially in cv. Arauco grown in La Rioja province. Using the MLST typing system, two sequence types (ST) exclusive to Argentina were identified; ST69 and ST78 being detected in olive and ST78 in almond. In this study the complete genomes of two strains of *Xf* subsp. *pauca* isolated from olive (La Rioja region) and one from almond (Catamarca region) were obtained using a hybrid assembly approach combining data from Illumina and Oxford Nanopore sequencing platforms. The assemblies were generated with Trycycler, polished with medaka + pilon and evaluated with BUSCO. All strains were assembled at the chromosomal level (~2.6 Mb) and one plasmid was found in each strain. A Maximum Likelihood tree including a worldwide collection of *Xf* genomes indicated a closer relationship of isolates from Argentina with those from Brazil. These genomes represent a valuable resource in comparative genomic studies to extend the knowledge of *Xf* outbreaks, providing data to determine potential *Xf* origin, virulence, and evolution. These are the first *Xf* genomes obtained from olive and almond trees in Argentina.

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20. UNDERSTANDING POTENTIAL VECTORS OF *XYLELLA FASTIDIOSA* IN AUSTRALIA

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Xylella fastidiosa although not present in Australia is a destructive plant pathogenic bacterium, with several subspecies affecting many plant species including grapevines, almonds, peaches, apricots, and olives in many countries worldwide. It is xylem limited, and transmitted mainly by spittlebugs, sharpshooters, leafhoppers and froghoppers. Xylella and its exotic vectors have been identified by the Plant Health Committee as the number one National Priority Plant Pest for Australia, by New Zealand MPI as an 'unwanted organism' and by the European Commission as one of most dangerous plant bacteria worldwide. Our research aims to provide biosecurity agencies with tools and knowledge which can be effectively implemented to rapidly eradicate *Xylella fastidiosa*, or prevent and suppress its spread if there is an incursion in Australia. This project focuses on potential insect vectors of Xylella, biology, physiology and ecology in targeted horticultural crops across three states of Australia. Efforts, methodology, results, and its significance to Australian food production will be communicated and discussed.

21. DECIPHERING XYLELLA POTENTIAL PATHOSYSTEMS IN THE BELGIAN FLORA: A STUDY ON NORTHERN EUROPEAN TEMPERATE REGIONS WITH EMPHASIS ON RIPARIAN AREAS

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**EFSA Young Researchers' Initiative*

The discovery of various strains belonging to three subspecies of *Xylella fastidiosa* in Europe has raised concerns about the potential spread of the bacteria to Northern regions. It is crucial to evaluate the susceptibility of European flora, which has not been exposed to this pathogen before. In a biosafety facility, we mechanically inoculated KLN59.3 GFP-labelled *X. fastidiosa* at 22°C and 28°C to assess the susceptibility of *Salicaceae*, including *Populus tremula, Populus canescens, Salix alba,* and *Salix caprea*. The movement and multiplication of bacteria in plants were examined using PCR, real-time PCR, confocal or scanning electron microscopy. After nine months, all plants tested positive for *X. fastidiosa*, except for 57% of *P. canescens* under the 22°C growing conditions. Bacteria were detected up to 120 cm from the inoculation point for *S. alba* and were found in the roots of all species. Successful isolation was achieved for *S. alba* and *P. tremula*. The average CFU/g of plant tissue per species ranged from 1.5E+03 to 3.5E+06, with the highest figures for *P. tremula*, which also showed a high number of totally obstructed vessels observed by confocal microscopy. Additionally, more than 400 endophytic bacterial isolates obtained from *Salicaceae* xylem sap, extracted using a Scholander pressure chamber, are currently under investigation as potential antagonist agents.

22. THE SECOND MESSENGER C-DI-GMP: VIRULENCE AND BIOFIM FORMATION BY *XYLELLA FASTIDIOSA*

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The vascular pathogen *Xylella fastidiosa* subsp. *fastidiosa* Temecula1 causes Pierce's Disease in grape plants. Bacterial proliferation blocks the transport of sap through the xylem, causing water and mineral nutrient alterations in the aerial parts of the plant. The pathogenicity mechanisms involve biofilm formation in the xylem vessels of infected plants. In the genome of Temecula1 there are few genes encoding GGDEF/EAL domain-containing proteins potentially involved in the turnover of c-di-GMP. It is known that this second messenger regulates the multicellular life style in bacteria. In *Xylella*, low levels of c-di-GMP are associated to acquisition of bacteria by the insect vector, while high levels of the second messenger are associated with the exploratory phase in plants (virulence). These phenotypes are contrary to the accepted canonical framework of c-di-GMP influencing the lifestyle of most phytopathogenic bacteria including other *Xanthomonadaceae*. We have generated mutants in GGDEF/EAL domain-containing proteins which were unavailable, investigated their role in virulence of grapevines and implemented a protocol to quantify c-di-GMP in these bacteria with the aim of certainly testing whether the c-di-GMP paradigm has an exception in *Xylella*, as it is suggested by the mutants' phenotypes. Identifying compounds which may inhibit biofilm formation by *Xylella fastidiosa* strains and unveil the mechanisms involved is also a goal.

23. USE OF APTAMERS AS A DIAGNOSTIC TOOL FOR *XYLELLA FASTIDIOSA*

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Aptamers are single-stranded nucleic acids capable of adopting defined three-dimensional structures that allow them to interact with high affinity and specificity with their target molecule. To identify aptamers that specifically and differentially recognize bacterial cells of *Xylella fastidiosa* (*Xf*), 12 rounds of selection against a mixture of five *Xf* strains belonging to different subspecies were performed, and counter-selection was carried out in all rounds against bacteria belonging to 20 genera that are common inhabitants of the plant xylem. The enrichment in *Xf*-specific aptamers of the populations obtained in successive rounds of selection was analyzed by real-time PCR and rounds 11 and 12 were cloned to obtain individual aptamers and analyzed by Next Generation Sequencing (NGS). The ability of six aptamers identified to recognize *Xf* bacteria cells was studied by slot-blot and ELONA and their structural characterization was performed with m-Fold (secondary structure) and QGRS mapper (possible G-quadruplex) software. Two aptamers have been identified (X11-5F and X136699R) able to recognize with a high affinity a set of 15 *Xf* strains belonging to four subspecies being their binding capacity significantly higher than that shown against other common xylem-inhabiting bacteria. The potential use of these aptamers as a diagnostic tool for *Xf* will be discussed.

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24. ESTABLISHMENT OF AN IN VITRO COLLECTION OF OLIVE CULTIVARS

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As part of the UN-programme for development of integrated techniques for inducing genetic diversity and improvement of vegetatively propagated and horticultural tree crops (CRP D24014) genotypes of Olea europaea were established in vitro. The research focused on developing and validate tissue culture protocols as a first step to reach induced stable mutants with tolerance to Olive Quick Decline Syndrome Disease. The cultivars 'Coratina', 'Favolosa' 'Frantoio', 'Leccino' and 'Ogliarola' were kept under greenhouse conditions. Single node explants of juvenile branches from trees were used for the initiation of the cultures. The aim of this work is to study the influence of factors such as explant maturity, position within the branch on the establishment of an in vitro shoot culture of *Olea europaea*.The basal medium Olive medium (OM) was supplemented with different cytokinins. Significant differences in the survival percentages were found between the cultivars with SPPS statistics 27. Interestingly, the response of explants from two different donor plants of 'Leccino' indicates the importance of the physiological state on the establishment process. Also explants from the two branch parts showed different survival rates with apical branch part performing better. However, significant differences were found in the shoot lengths after three months. Here explants from the basal branch part produced significant longer shoots in most cultivars.

25. APPLICATION OF A WEB-BASED APP (CLASNIP:WWW.CLASNIP.COM) FOR THE IDENTIFICATION AND CLASSIFICATION OF FASTIDIOUS PLANT PATHOGENS

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Fastidious plant pathogenic bacteria inhabit either the xylem or phloem of the vascular tissues of plants. They are usually transmitted from plant to plant by insect vectors, which also serve as hosts for reproduction and overwintering. These pathogens either resist to growth in any available media such as '*Candidatus* Liberibacter solanacearum' (CLso), or require specific and enriched media such as *Xylella fastidiosa* (Xf), which makes identification and classification challenging. Here we introduce Clasnip (www.clasnip.com), a web-based platform implemented with a state-of-art classification algorithm by deriving the Maximum Likelihood estimation of the Hidden Markov Model (HMM) parameters, for rapid and reliable interspecific and intraspecific interpretation of sequences reads. To date, Clasnip has newly curated databases for the classification of potato zebra chip (CLso), grapevine pierce disease (Xf), bacterial ring rot (*Clavibacter sepedonicus*), soft rot and blackleg pathogens such as *Dickeya* and *Pectobacterium* spp., and potato virus Y phylogroups. We will demonstrate the high accuracy of Clasnip in classifying CLso haplotypes and Xf subspecies from pure culture and environmental samples using Sanger, genome and metagenomics sequencing data. This intuitive platform also allows users to customize their own reference databases for expanded detection coverage. Clasnip is a decision-making tool that can facilitate disease containment and control disease outbreaks or invasions.

26. IMPROVING PLANKTONIC GROWTH OF *XYLELLA FASTIDIOSA* SUBSP. *PAUCA*, STRAIN DE DONNO BY SUPPLEMENTING LIQUID MEDIA WITH POLYSORBATE SURFACTANTS

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Xylella fastidiosa (*Xf*) is a fastidious and slow growing bacterium, difficult to grow *in vitro*. Isolates of *X. fastidiosa* subsp. *pauca* (*Xfp*) recovered from diseased olive trees in Apulia (Italy), are notoriously difficult to be cultivated in common media (PD3 and PW or PWG), and growth in liquid cultures is very limited, most likely for the abundant formation of biofilm that promotes the aggregation of the bacterial cells, forming the typical and visible ring of biofilm and cell aggregates. Such behaviour poses major constrains for testing the effects of different growth conditions or compounds on the bacterial multiplication. In this study, we tested the effect of two polysorbate surfactants, Tween-20 (TW20) and Tween-80 (TW80) at different concentrations, on the growth and biofilm formation of *Xfp* in different liquid media. TW20 and TW80 are widely used in microbiology, biochemistry, and molecular biology due to their excellent solubilization properties and low toxicity. Our results showed that TW20 positively affects the planktonic growth of *Xfp* and reduce the biofilm formation. Whereas, when TW80 was used an increase of the planktonic growth rate was recorded, but not a decrease in the biofilm formation. Overall, our results showed that by adding the two surfactants, *Xfp* planktonic growth can be significantly increased, allowing to improve *in vitro* tests aimed at evaluating the inhibition effects of compounds and antimicrobial formulations.

27. INTERPLAY BETWEEN BIOTIC AND ABIOTIC STRESS EXACERBATES LEAF SCORCH DEVELOPMENT IN ARABIDOPSIS INFECTED WITH *XYLELLA FASTIDIOSA*

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Abiotic stresses, as drought, can induce xylemic vessel emboly and loss of water potential. While these responses are essential for plant survival during drought, some bacteria may evolve to explore these host responses for their own gain. *Xylella fastidiosa*, a vector borne xylem pathogen, specifically colonizes more than 500 different plant species. Through feeding, insects transmit the bacterium to the xylem. Xylella cells form a biofilm attached to the cell walls. Interestingly, within these plant species some develop disease symptoms, as leaf scorch, while others remain symptomless. The underlining molecular mechanisms, and the relative importance of the host response to the abiotic stress vs biotic stress as of outcome for bacterial growth and/or disease development remains unexplored. Here, we investigated the effect of drought stress on the establishment and development of symptoms in Arabidopsis infected with Xylella. Plants were exposed to drought stress for 10 days after Xylella infection. 75% of the Xylella infected leaves show rapid development of symptoms after watering, compared to either, mock infected, or normally raised Xylella infected leaves. This effect was independent of the Xylella subspecies used. Our data suggests that the host response to drought stress may increase the ability of Xylella to colonize the host. Studying the interface between drought and infection may reveal useful molecular mechanisms to create plants resistant to infection.

28. SCREENING AN ALMOND GENOTYPE COLLECTION FOR RESISTANCE TO *XYLELLA FASTIDIOSA* AND IDENTIFYING DNA MARKERS ASSOCIATED WITH THIS TRAIT

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Xylella fastidiosa causes almond leaf scorch (ALS) disease in North America, the EU and the Mediterranean. Controlling the disease once trees have been infected is practically impossible, and therefore finding resistant genotypes can be an elegant solution to reduce ALS damage. We have recently identified a resistant almond genotype by conducting a small-scale screen of our almond genotype collection. In the current study we have expanded this screen to test 50 different almond genotypes. From each genotype 8 seedlings were generated by grafting genotype scions on GF677 rootstocks. Seedlings will be inoculated with *X. fastidiosa* using the needle prick method during the spring and evaluated for disease incidence, severity, and *X. fastidiosa* colonization during the summer. The Unit of Fruit Tree Sciences in Newe Yaar has established a map of ~5000 SNP markers, evenly spread across the genome of these almond genotypes. Using this map, the Unit of Fruit Tree Sciences will lead the research to link *X. fastidiosa* resistance to specific DNA markers, which represent a region of approximately 40K bp. This will allow to identify not only markers linked to almond resistance but also candidate genes which may be relevant for the resistance phenotype. Hence, results from these experiments are expected to advance breeding efforts to generate *X. fastidiosa* resistant almond cultivars and to help understand the genetic basis of resistance to ALS.

29. EVALUATION OF MOLECULAR TESTS FOR THE DETERMINATION OF THE *XYLELLA FASTIDIOSA* SUBSPECIES: RESULTS FROM AN EUROPEAN TEST PERFORMANCE STUDY (TPS) AND THE ORGANIZATION OF A PROFICIENCY TEST (PT)

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The European Commission has identified *Xylella fastidiosa* (Xf) as a priority pest to be addressed in the activities of the European Union Reference Laboratory for Pests of Plants on Bacteria (EURL). The detection of Xf has been harmonized among the national reference laboratories (NRLs). Currently, Xf subspecies determination requires a laborious multilocus sequence typing (MLST) test (Annex IV B of Commission Implementing Regulation (EU) 2020/1201). Over the past few years, two real-time PCR tests have been published that focus on the Xf subspecies designation. These real-time PCRs offer several advantages when compared with the implemented MLST test: firstly, they should increase the sensitivity of detection, and secondly, they should decrease the time required for subspecies designation. However, available validation data is currently rather limited. Therefore, the EURL (Netherlands Institute for Vectors, Invasive plants and Plant health-NIVIP) has scheduled a TPS on the Xf subspecies determination by these real-time PCRs. The TPS consists of 38 blind samples containing DNA from different Xf subspecies (either mixed with plant DNA or in buffer), or plant DNA and 6 controls. The results obtained from this TPS will be discussed, as well as the organisation of a PT on the subspecies designation of Xf in 2023. Results will support the addition of one or both real-time PCR tests to the Annex IV B of Commission Implementing Regulation (EU) 2020/1201.

30. HOW EUROPEAN VINEYARDS ESCAPED PIERCE'S DISEASE DURING THE XIX AND XX CENTURY

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Pierce's disease (PD) of grapevine caused by *Xylella fastidiosa* (Xf) exhibits a limited global distribution compared to other grapevine diseases, e.g. powdery and downy mildews. Why European vineyards were not affected by PD during the 19th and 20th centuries is a matter of scientific debate, but no clear answer has yet been provided. Here we gather phylogenetic, epidemiological, and historical evidence indicating that (i) the spread of Xf in vineyards of the southeastern USA likely occurred after the *Phylloxera* crisis in Europe (~1868-1890) and that (ii) most of American-vine species exported to Europe came from nurseries located in areas without PD risk in the USA, according to our epidemiological model. Once in France, American vines were multiplied and distributed mainly from Montpellier and Bordeaux. Using reanalysed temperature data from 1950 to 1980 we show how Montpellier would be located in an epidemic-risk zone with potentially low to very low growth rates, while temperatures between 1870 and 1900 would be below the threshold for PD epidemics (T_{av} ~ 0.5ºC lower). Our temperature-driven epidemiological model suggests moderate PD risks only in Mediterranean islands and some coastlands areas before 1980. However, global warming is accelerating since 1990 the extent and risk levels of PD in continental areas of the Mediterranean basin (e.g. Rhone valley, central Italy) and the Atlantic face of Portugal and southern France.

31. NANOPORE AMPLICON SEQUENCING: RAPID, SENSITIVE AND SPECIFIC DIAGNOSTIC SYSTEM TO MONITOR AND INTERCEPT *XYLELLA FASTIDIOSA*

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Xylella fastidiosa (Xf) is a quarantine bacterium causing important diseases in various host plants. Since 2013 it has been intercepted in several European countries, starting from Italy. The recent rise in minimum winter temperatures, due to climate change, has extended the range of areas suitable for its establishment, in fact cold temperature is one of the main deterring factors for Xf establishment. The spread of this bacterium often comes from asymptomatic plant materials for which sensitive detection is needed. Moreover, in case of new outbreak or new host plant, the identification of the subspecies and the Sequence Type (ST) is required. With this purpose, an amplicon-Nanopore sequencing was developed for Xf detection and identification at the subspecie/ST level, even in asymptomatic conditions. The workflow foresees the amplification of seven housekeeping genes (MLST) and the sequencing based on MinION from Oxford Nanopore Technology, a portable, fast and easy-to-use device, paired with an ad hoc bioinformatics pipeline. Two host plants, grape and olive, have been spiked with the Xf subspecies fastidiosa and pauca respectively, at different known concentrations (from 10^7 to 10 cfu/mL). Preliminary results indicate that this approach is a promising tool to precisely detect and identify Xf and therefore can be used to monitor the spread and the evolution of this bacterium. Finally this workflow could be applied for the correct identification of other priority pathogens.

32. GENETIC VARIATION WITHIN ALMOND POPULATIONS DO NOT IMPEDE LEAF SCORCH OUTBREAK IN MALLORCA

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Genetic variation within host populations decreases disease risk and spread in annual crops, but this central prediction in evolutionary ecology remains largely untested in perennial crops. Around 30 years ago, two subspecies of *Xylella fastidiosa* (Xf) were introduced to the island of Majorca and subsequently dispersed by the insect vector, *Philaenus spumarius*, mainly in almond crops. During this time, almond germplasm banks distributed in various locations on the island have been naturally exposed to the pathogen. Between 2019 and 2021, the symptoms of 95 mostly local varieties of almond trees exposed to the pathogen were analyzed and monitored each year in the germplasm collections. Xf have been detected in 82 of the 95 almond varieties analyzed. In general, a good correspondence was observed between varietal response to the pathogen in the germplasm almond collection and in the field. Despite the great spatial and genetic heterogeneity found in Mallorcan almond orchards, Xf has been effectively transmitted among almond trees and orchards, causing almond leaf scorch disease in more than 1.5 million trees (83% incidence) across the island. Analysis of the data suggests that although transmission rates may be higher in host populations with genetic uniformity, vector movement is sufficient to bridge the spatial gaps between orchards containing with more resistant varieties.

33. SURVEYS ON *XYLELLA FASTIDIOSA* AND ITS VECTORS IN MONTENEGRO

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Xylella fastidiosa (Xf) causes various diseases on important crops such as olives, grapevines, citrus fruits, almonds, but also forest trees, ornamental plants and plants of spontaneous flora. Since the olive is the most widespread fruit species in Montenegro, possible presence of the Xf bacterium would cause great economic losses. The paper presents the results of a three-year survey of olive trees and other host plants carried out in olive groves, nurseries, home and public gardens. With visual inspections a special attention was paid to the presence of typical and atypical symtoms. A total of 120 samples were colected and analyzed by the LAMP method (Yaseen et al., 2015), dominantly *Olea europaea* (39.2%), followed by *Citrus* spp., *Prunus* spp., *Nerium oleander*, *Laurus nobilis*, *Magnolia grandiflora*, *Rosmarinum officinalis* and other host plant. In the analyzed plant material phytopathogenic bacteria Xf was not detected. Bearing in mind the importance of vectors in the transmission of Xf from infected host plants to healthy ones the presence of insect vectors in olive groves on the Montenegrin coast was monitored as well. Insects were collected on weeds, using sweep-net sampling at three locations in the area of Valdanos and two locations in the area of Radanovici. The insect population in the area of Valadanos compared to population in Radanovici was richer in number of species and more abundant in number of individuals.

34. A PHYSIOLOGICALLY-BASED POPULATION MODEL OF *PHILAENUS SPUMARIUS*

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The meadow spittlebug, *Philaenus spumarius* L. (1758) (Hemiptera: Aphrophoridae), is the main vector of the plant pathogen bacterium *Xylella fastidiosa* in Europe. Effective disease containment strategies are mainly based on uprooting infected plants and control of the vectors. The vector control strategy focuses on suppressing juveniles in the herbaceous cover and preventing colonization of the olive canopy by newly-emerged adults. The design and implementation of vector control strategies can take advantage of properly calibrated population models describing and predicting the phenology and abundance of *P. spumarius* populations in agroecosystems.

We developed a temperature-driven physiological-based model to predict the phenology and population dynamics of *P. spumarius*. We parametrized model functions describing the diapause termination and age-distribution of overwintering individuals, and the temperature-dependent development and mortality rates by integrating data collected in lab experiments and literature. The model has been calibrated and validated with field data collected in Northern and Southern Italy (in the Liguria and Apulia regions).

The model can be used for a proper definition of effective Integrated Pest Management strategies to control *P. spumarius* populations and thus to support *Xylella fastidiosa* containment.

35. CHARACTERIZATION OF THE AMYLOIDOGENICITY OF REP-WH1 DOMAINS IN PLASMIDS FROM *XYLELLA FASTIDIOSA*

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We have been working for many years with a plasmid replication protein from *Pseudomonas savastanoi* (RepA), achieving its structural and functional characterization. Such knowledge has lent to prove that its WH1 dimerization domain has amyloidogenic traits. Thus, we were able to trigger the formation of amyloid aggregates, eliciting toxicity in *E. coli* by targeting the bacterial inner membrane, disrupting PMF and hampering transport through membranes, central metabolism and ATP/nucleotide synthesis and generating ROS.

Here we report the use of the homologue Rep-WH1 domains from two plasmids of *Xylella fastidiosa* (*spp. pauca* and *multiplex*) to trigger an amyloid proteinopathy. In *E. coli*, depending on the Rep-WH1 variant, it forms distinct amyloid aggregates (according to Th-S staining), drastically changing bacterial morphology, growth rates and viability. DnaK (Hsp70) chaperone overexpression, which shifts the aggregative behaviour of *P. savastanoi* RepA-WH1[5,6], also altered the aggregation pattern in one of the variants from *X. fastidosa*. Our goal is to use these Rep-WH1 variants as a control strategy against *X. fastidiosa*.

36. EPPO DIAGNOSTIC PROTOCOL ON *XYLELLA FASTIDIOSA*: HOW NATIONAL AND TRANSNATIONAL RESEARCH FEED INTO REGIONAL STANDARDS

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EPPO is one of the Regional Plant Protection Organizations recognized under the International Plant Protection Convention. One of its functions is to develop Regional Standards. EPPO has a long standing and active program for Standard setting in several areas and started a program to prepare diagnostic Standards in 1998. The objective of this program is to achieve a harmonized approach to detection and identification for regulated pests. The work is conducted by the Panel on Diagnostics and Quality Assurance in collaboration with specialized Panels (including one in Bacteriology). Panels are composed of specialists from member countries. As of March 2023, 145 Pest-specific diagnostic protocols and horizontal diagnostic Standards have been developed. The first version of the Diagnostic protocol on *Xylella fastidiosa* (Xf) was approved in 2003 and focused on Vitis and Citrus hosts. After the introduction of the bacterium in Italy a first revision of the protocol was prepared to include information on the different hosts of Xf (including symptoms), details on sampling and sample preparation and new tests for detection and identification. Further revisions have been initiated to incorporate outcomes of transnational research projects (e.g. Euphresco PROMODE, POnTE and XFActors). The fifth version of the diagnostic protocol was approved in 2023-02. The EPPO Secretariat is a partner in BeXyl and relevant outcomes will be considered for inclusion in the EPPO protocol.

37. COMMUNITY STRUCTURE AND SEASONAL ABUNDANCE OF POTENTIAL *XYLELLA FASTIDIOSA* VECTORS AND RELATED TAXA IN XYLELLA-SUSCEPTIBLE CROPS IN SOUTHERN NEW SOUTH WALES, AUSTRALIA

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Xylella fastidiosa (*Xf*) and its vectors (xylem-feeding leafhoppers and spittlebugs) are considered the single greatest threat to Australian plant biosecurity and Australian horticultural industries. Despite this, potential native vectors of *XF* in vulnerable crops in Australia are unknown. Thus, the aim of our study is to examine the diversity and seasonal abundance of leafhoppers and spittlebugs present in *XF* vulnerable crops and to discover if any have the potential to be vectors of *XF*. We did this by trapping and sweep netting, every two to three weeks, in *XF* vulnerable crops (wine grapes, citrus, olives and cherries) and surrounding native vegetation in one of the main horticultural areas of southern NSW. The results of this trapping effort and possible implications for the transmission of *XF* and other plant pathogens in Australia are discussed here.

38. THE POTENTIAL DIRECT ECONOMIC IMPACT AND PRIVATE MANAGEMENT COSTS OF AN INVASIVE ALIEN SPECIES: *XYLELLA FASTIDIOSA* ON LEBANESE WINE GRAPES

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Xylella fastidiosa subsp. *fastidiosa* (Xf) has not been reported in Lebanese vineyards. The average gross revenue generated by Lebanese wine growers is estimated as close to US$22 million/year for 2015-2019. The potential quantitative economic impacts of an Xf outbreak and particularly, the private control costs have not been assessed yet for this country as well as for others which Xf may invade. Using Partial Budget approach at the farm gate, we estimated that a hypothetical full spread of Xf on Lebanese wine grapes would lead to maximum potential gross revenue losses of almost US$ 11 million for an average recovery period of 4 years, to around US$ 82.44 million for an average grapevine life span period of 30 years in which infected plants are not replaced at all. The first yearly estimated additional management cost is US$853 per potentially infected hectare. For a recovery period of 4 years, the aggregate estimated additional cost would reach US$2374/ha, while the aggregate net change in profit would be US$-4046/ha. The observed costs in this study support the concerned policy makers and stakeholders to implement a set of reduction management options against Xf at both national and wine growers' levels. This re-emerging alien biota should not be neglected in this country. This understanding of the potential direct economic impact of Xf and the private management costs can also benefit further larger-scale studies covering other potential infection areas and plant hosts.

39. PHILAENUS SPUMARIUS VIROSPHERE: THE BEXYL PROJECT

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The use of virus-based biocontrol technologies as sustainable alternatives to pesticides in pest management has been extensively discussed for decades. The application of next-generation sequencing (NGS) to viral metagenomic analyses of insect populations has significantly increased the discovery rate of viruses, leading to the identification of new virus-based tools for pest management. In this perspective, the Horizon Europe-funded BeXyl project includes a task, within the work package 5, which aims at identifying potential biocontrol agents from the virosphere of the *Xylella fastidiosa* vector *Philaenus spumarius* (Hemiptera: Aphrophoridae). Eight European *P. spumarius* populations were sampled from fields characterized by distinct ecological traits, and their transcriptomes were analyzed using NGS. In this work, we present the preliminary composition of the European virome assigned to *P. spumarius*. Beside the evaluation of potential pathogenic viruses to be exploited as bio control agent, the most recurrent viruses will be further characterized alone or in combination with other stress factors to ascertain if they produce fitness cost for the vector and can be regarded as potential tools in an integrated pest management perspective. In conclusion, the identification and characterization of virus-based biocontrol agents can contribute to the development of more efficient and environmentally friendly pest management strategies.

40. CHARACTERIZATION OF THE METABOLIC DIVERSITY OF *XYLELLA FASTIDIOSA* STRAINS BY HIGH-THROUGHPUT PHENOTYPING

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**EFSA Young Researchers' Initiative*

Xylella fastidiosa (*Xf*) is a fastidious-growing bacterium with a wide range of host plants, being able to live with a commensal or pathogenic life style in hundreds of plant species depending on the association among specific *Xf* strains and host genotype. *Xf* phenomic characterization is key to establish potential alternative or adaptative metabolic pathways changes on certain host plants. A metabolic network using the sequences of 22 different *Xf*-strains has been proposed, and a high-throughput phenotyping approach was used to characterize the metabolic fingerprinting of seven *Xf* strains belonging to the three main subspecies isolated from several hosts. The number of carbon sources used ranged from 35 to 75, while compounds used as nitrogen sources varied from 12 to 37; with only between 14 and 22 % of the compounds being used by all the strains studied. The model developed with the *Xf* core gene set identified several key functions and the results obtained with the phenotyping microplates supported the proposed metabolic network. Different new defined minimal media have been designed based on the metabolic network and results from metabolic fingerprinting indicating that growth and biofilm formation differed according to the type of carbon and nitrogen source used, although in general all strains had lower growth compared to that obtained in PD3 medium.

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41. THE EPPO INSPECTION STANDARDS FOR *XYLELLA FASTIDIOSA*: HOW NATIONAL AND TRANSNATIONAL RESEARCH FEED INTO REGIONAL STANDARDS

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EPPO is one of the Regional Plant Protection Organizations recognized under the International Plant Protection Convention. One of its functions is to develop Regional Standards. EPPO has a long standing and active program for Standard setting in several areas including inspection. Inspection Standards are developed to provide information to phytosanitary inspectors on when and how to inspect consignments or places of production of plants for planting and include sampling guidance (how lots are identified, and what is the unit of inspection). The EPPO Inspection Standards PM 3/81(3) Inspection of consignments for *Xylella fastidiosa* and PM 3/82(3) Inspection of places of production for *X. fastidiosa* were developed by the EPPO Panel on Phytosanitary Inspections following the introduction of *X. fastidiosa* in the EPPO region. Both Standards, were first approved in 2016 and have been revised twice, with the most recent update in 2022 incorporating additional sampling guidance (e.g. pooling of samples) from the EU H2020 project XF-ACTORS. Both Standards include common sections, e.g. information on vectors of *X. fastidiosa*, host plants, symptom description and general elements for phytosanitary inspections. The EPPO Secretariat is a partner in BeXyl and relevant outcomes will be considered for inclusion in the EPPO Standards.

42. DYNAMIC MODEL OF THE *XYLELLA FASTIDIOSA* PATHOSYSTEM: AN EXAMPLE ON ALMOND TREES FROM SOUTH-EAST SPAIN

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The almond tree is one of the most widely grown species around the Mediterranean basin, in southern Spain alone it generates an estimated net economic impact of €9 billion/y. However, since 2016, the xylem-feeder bacteria *Xylella fastidiosa* has spread and infected crops from the Balearic Islands to peninsular Spain. The main transmission vectors of *X. fastidiosa* in the Spanish Mediterranean are the spittlebugs *Philaenus spumarius* and *Neophilaenus campestris*. Despite all the management measures taken, the situation has become critical in certain areas, such as the northern part of the province of Alicante, Spain.

As an alternative to the institutionally-sanctioned use of pesticides and crop eradication campaigns, other strategies which are currently in preliminary experimental stages are being sought out that directly target the pathogen stages. One such approach currently being investigated in our laboratory, involves the use of antibacterial endolysins, which are peptidoglycan-lysing enzymes derived from bacterial viruses.

To support these efforts, we present a dynamic model of the entire *X. fastidiosa* pathosystem. Our model is based on field data and literature. This model allows us to develop simulations of diverse scenarios, which help to identify where different measures should be implemented. By incorporating laboratory data, our results show a first insight into the required effectiveness of lysins to control the spread of the bacteria.

43. *XYLELLA FASTIDIOSA* AFFECTS THE ALMOND TREES IN THE PROVINCE OF ALICANTE (SPAIN). SINCE WHEN?

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In 2017, *Xylella fastidiosa* (Xf) reaches the almond trees of the province of Alicante (Spain). Our project (AICO 2021/331) funded by valencian government consists of developing mathematical-computational models that incorporate environmental variables. For validation, our model representing the spread of the plant pest must be fitted to the actual data available. Here we ran into a difficulty, because we are not sure the date of the analysis of the samples coincides with the date on which the tree was infected. The origin (time and coordinates) of the infection is unknown. So, the question is, when and where did the contagion of the almond trees really begin in the province of Alicante?

Our hypothesis is Xf has been around long before 2017. It is based on the observation and analysis of the production of almonds both in the province of Alicante and in Spain. Since almost twenty years ago, we observe a decline in almond production and cultivated area due to abandonment by farmers that suffer low crop yields, while production has increased in other areas in Spain. Despite new plantations have a higher yield, the permanent decrease in the harvest confirms the unstoppable destruction, if not remedied, of the traditional Valencian almond tree.

Although there are several causes such as inclement weather, scant help from agricultural insurance and disproportionate increase in production costs, the downward trend being almost 50% in some varieties could confirm our hypothesis.

44. POTENTIAL ALTERNATIVE WOODY PLANTS FOR THE RENEWAL OF "POST-XYLELLA" AGRICULTURE IN SALENTO (ITALY)

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**EFSA Young Researchers' Initiative*

Since its first discovery in 2013, the epidemic of *Xylella fastidiosa* subps. *pauca* ST53 in few years devastated the olive orchards of 3 provinces in Salento (Puglia, Italy) causing huge economic, landscape and environmental problems as well raising the need to rebuild the main and almost unique arboreous coverage, increasing biodiversity and resilience of the agro-ecosystem. The identification/evaluation of alternative woody agricultural and agro-forestry crops/species, both native and potentially adaptable to the soil/climatic conditions of Salento, is the starting point to regenerate and reconvert the future agriculture living together the bacterium. In the framework of a wider national project, the research is aimed to study the main aspects of alternative plants in term of agronomic requirements, characteristics, cultivation, protection, uses, investments and market. The information will be available as a "reasoned catalogue" that draws data/info from bibliographical research, specialist consulting, small farming experiences and pilot projects intercepted in the infected area. Furthermore, for the species not present in Salento, pathogenicity tests will be carried out, by controlled artificial/vector inoculation and periodic qPCR analyses, to verify their immunity or the level of resistance of the selected species. The results are addressed to producers/technicians to guide investments or pilot field trials as well to policy makers to plan strategies and funding measures.

45. KNOWLEDGE ON VECTOR'S FAUNA FOR *XYLELLA FASTIDIOSA* INVASION PREPAREDNESS: XYLEM FEEDERS IN ALBANIAN OLIVE ORCHARDS

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**EFSA Young Researchers' Initiative*

Since its first occurrence in Southern Italy, *Xylella fastidiosa* (hereafter Xf) has gained importance, especially in matters of its management and limiting its transmission. In regions non-affected by the bacterium, such as Albania, it is necessary to assess the risks of spread, notably by understanding the ecology of its vectors. Surveys took place over 12 months from August 2015 and July 2016 in two Albanian regions (Vlora and Tirana), where olive production is important covering 14 olive orchards. The aim was to investigate the presence of Auchenorrhyncha species with particular attention to xylem feeders. Identified Auchenorrhyncha species belonging to families Aphrophoridae, Cercopidae, Cicadellidae, Issidae, Flatidae were found. In Vlora, 4 out of 11 species captured were xylem feeders, whereas, in Tirana, 3 species were captured. The most abundant xylem feeder species was *Philaenus spumarius* in both regions. Vlora showcased a higher presence of *P. spumarius* in olive trees than in Tirana. Adults of *P. spumarius* and *Neophilaenus campestris* were the most frequent in August, September, and October 2015. The agroecological analysis noted that the Vlora area shows a higher richness of Auchenorrhyncha in terms of the number of species. Further, the analysis of real-time Lamp assay confirmed the absence of Xf in the tested individuals. This study has public implications and pointed out that this country is still a free territory from Xf, at least in the surveyed areas.

46. TRANSLOCATION OF QUORUM SENSING MOLECULES FROM TRANSGENIC ROOTSTOCKS TO NON-TRANSGENIC SWEET ORANGE SCIONS INCREASES RESISTANCE AGAINST BACTERIAL DISEASES

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One of the greatest challenges for sustainable food production is the decrease of productivity due to pathogens, which makes urgent studies on plant-pathogen interactions regarding disease management. Previously, we developed a genetically modified citrus variety, carrying rpfF gene from *Xylella fastidiosa*, which was able to produce diffusible signaling factor (DSF), a quorum sensing molecule, and cause “pathogen confusion” to *X. fastidiosa* and *Xanthomonas citri*, thus attenuating CVC and citrus canker (CC) symptoms. Currently, we are investigating whether RpfF-expressing GM rootstocks could translocate DSF and improve the disease tolerance to non-transgenic scions. Hence, we verified that RpfF was produced at high levels in “Carrizo” rootstocks and triggered tolerance to CC in non-transgenic scions. Based on this, we also transformed “Swingle" rootstock, resulting in 3 GM lines (GM-SR1- 3), onto which non-GM “Valencia” sweet orange (NGM-SO) was grafted. To prove DSF translocation, *X. citri* was used as a biosensor due to its rapid infection process. Thus, leaves from each NGM-SO/GM-SR and control (NGM-SO/NGM rootstock) were challenged with *X. citri*-GFP. Genes regulated by DSF were monitored through RT-qPCR. The lines showed significantly lower symptoms and bacterial titer compared to the control. Furthermore, significant modulation of DSF-responsive genes demonstrated the potential use of GM-rootstocks to translocate DSF and confer disease tolerance to non-transgenic scions.

47. ACHIEVEMENTS, CHALLENGES AND FUTURE PERSPECTIVES IN CONTROLLING THE MENACE OF *XYLELLA FASTIDIOSA* BY ORGANIC NANOCOMPOUNDS – THE CASE OF ANCOSIX PROJECT

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Xylella fastidiosa (*Xf*) is a Gram-negative bacterium that causes devastating diseases in a wide range of economically important crops, including olives, almonds, grapes, citrus, and many others. Europe has been specifically hit in the form of Olive Quick Decline Syndrome since 2013, which has drastically changed the historical Olive landscape in Italy and other EU states as well. Among the various proposed counteract methods, only few of them have been resulted effective against the bacterium: controlling insect vectors, monitoring wild hosts, following good agronomical practices to keep the orchards healthy, rapid detection methods, screening of tolerant and resistant genotypes, eradication of diseased plants. However novel approaches are being investigated for their potential application in field, such as RNA interference, satellite imagery, phages and the use xylem-located nanomaterials. Considering our previously achieved results in which the hydroalcoholic pomegranate peels' extract at 0.02 g/mL (patent no. TO2013A001085) and cellulose nanocrystals at 0.01 g/mL were able to reduce the *in vitro* planktonic growth and biofilm synthesis of two *Xf* strains (NCPPB4605 and CFBP8402), we developed an experimental project to formulate a novel nano-compound made from the two substances. ANCOSIX project is funded by the Italian Ministry for Agriculture (MASAF) to find sustainable ways to deliver the proposed nanopesticide directly to in field diseased plants and study its effects on *Xf* infection.

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48. HOW TO COMBINE DETECTION AND IDENTIFICATION METHODS TO MAXIMISE THE METHOD SENSITIVITY FOR THE SURVEILLANCE OF *XYLELLA FASTIDIOSA*

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Since 2017, EFSA assists EU Member States in the preparation, design and reporting of statistically sound and risk-based surveys of *Xylella fastidiosa* through a pest survey card and specific survey guidelines. One of the core sections included in *X. fastidiosa* pest survey card is how to detect and identify the pest to set the overall method sensitivity. This measure is a key parameter in the survey design, and it is an estimation resulting from sampling effectiveness and diagnostic sensitivity. The method sensitivity can be increased by selecting and integrating a set of different detection and identification methods to be applied in the field and in the laboratory. In fact, the success of X. fastidiosa detection and identification depends on the combination of visual examination, sampling and testing of plant material. Remote sensing and hyperspectral imaging techniques can reveal risk areas for focussing visual examination of symptoms on host plants. In the identified risk area, plant samples can then be collected from both symptomatic and asymptomatic host plants: this would improve the performance of the detection compared to a fully randomised sampling of plant material. The samples are eventually tested in the laboratory following validated molecular and serological tests. This poster will provide examples and comparisons of combinations of different detection and identification methods to choose the one with the highest method sensitivity for X. fastidiosa surveys.

49. ENHANCING PCR DETECTION OF *XYLELLA TAIWANENSIS* USING WHOLE GENOME SEQUENCE INFORMATION

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Xylella taiwanensis (Xt) is a nutritionally fastidious bacterial pathogen causing pear leaf scorch disease (PLSD) in Taiwan. Xt detection plays a key role in PLSD management. Multiple PCR systems based on single- or two-copy genes have been developed for Xt diagnosis. Xt detection could be further improved utilizing multi-copy genes. A total of 32 Xt whole genome sequences are now available in the GenBank, providing a sound resource for the development of robust PCR detection systems. By self-aligning the genome sequences of Xt Type strain PLS229, seven 714-bp sequences with similarity > 97% each other were identified. The sequences were annotated as part of the hemagglutinin-like protein gene. Based on this sequence, two primer sets, Xt7cp-378-F/R and Xt7cp-573-F/R, were designed. In silico experiment using GenBank database showed the high Xf-specificity of the two primer sets. SYBR green qPCR experiments using pure culture DNA from strains of Xt, *X. fastidiosa* and *Xanthomonas campestris* and PLSD plant DNA samples collected in Taiwan further confirmed the Xt-specificity. To evaluate PCR detection sensitivity, five previously developed Xt-specific primer sets, two from single-copy locus and three from two-copy locus, were simultaneously compared with Xt7cp-378-F/R and Xt7cp-573-F/R against the same set of PLSD samples. A reduction of 1-3 Ct values from the two 7-copy gene-based PCR systems were observed. Further evaluation of the two PCR systems is underway.

50. BIOVEXO - BIOCONTROL OF XYLELLA AND ITS VECTOR IN OLIVE TREES FOR INTEGRATED PEST MANAGEMENT.

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Xylella fastidiosa is increasingly causing diseases in olive trees and various other crops in the Mediterranean region. It has wiped out a number of olive and almond groves in Italy and Spain in only a few years. Unfortunately, the climate of the southern European Union is ideal for *Xylella*. Due to its rapid transmission across cultivation areas, *Xylella fastidiosa* is projected to cause yield losses of 35% - 70% in olive harvests and 13% in almond harvests. Currently, there are no products available on the market that have been proven to be effective against *Xylella fastidiosa*, which is spread by xylem sap-feeding insects – notably the spittlebug *Philaenus spumarius*. Some chemical insecticides are authorized to control xylem-feeding insects; while for products allowed in organic farming, only temporary authorizations, for limited periods, have been obtained. In the BBI JU-H2020 project BIOVEXO (1), 11 partners from 5 different countries and belonging to industries, SMEs, RTOs, and universities aim to develop environmentally sustainable and economically viable plant protection solutions that can be deployed as a method of integrated pest management to control *Xylella fastidiosa* and its spittlebug vector. After 2.5 years of BIOVEXO, both V-biopesticides and X-biopesticides have been upscaled and formulated and are currently being applied via endotherapy, foliar spray, or by drip irrigation on large scale field trials (olive and almond trees) as preventive and curative approaches, and also in integrated management, in Italy (Apulia) and Spain (Alicante and Mallorca). The biopesticides' mechanisms of actions are also being investigated and this investigation considers plant transcriptome approaches, plant and insect microbiomes, as well as colonization and persistence of the biopesticides in the insect/plant-environment. Toxicity and life cycles of each biopesticide are also being assessed. At the end of BIOVEXO, the two best performing solutions will be brought closer to the market (Technical Readiness Level 7-8).

For more information about the BIOVEXO project: www.biovexo.eu.

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51. BEXYL - BEYOND XYLELLA, INTEGRATED MANAGEMENT STRATEGIES FOR MITIGATING *XYLELLA FASTIDIOSA* IMPACT IN EUROPE

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BeXyl will contribute to promote research and innovation and specifically provide interdisciplinary solutions according to the demand of the stakeholders embebed in the project, contributing to deliver specific impacts for each of the expected outcomes as foreseen by the HORIZON-CL6-2021-FARM2FORK-01-04 call. In line with the conceptual approach of the new EU Plant Health Law, BeXyl has been conceived to strengthen the two pillars of plant protection: i) prevention and ii) control strategies for the priority quarantine pathogen *Xylella fastidiosa* (Xf). The workplan is designed to assess the risk of new Xf outbreaks in the EU under changing climate (WP1), improve the methods for border inspections and surveillance for early detection (WP2), ensure the phytosanitary standards for the trade of plant propagation materials using non-chemical treatments (WP3), restore outbreak areas based on the strategies to improve host plant resistance (WP4) and disease management tools (i.e., Decision Support Systems and IPM protocols) in conventional and organic production (WP7) based on investigations for the control of insect vector populations (WP5) and innovative biological tools for Xf (WP6). Outbreak management approaches will take fully into account the ecological and socioeconomic contexts for their adoption and implementation (WP8). In line with the EU Green Deal for a transition to environmentally sustainable, healthy and resilient agriculture and forestry, BeXyl will support research, innovation and transfer of knowledge (WP9) for crisis preparedness to help to prevent new Xf outbreaks and further spread of currently infested areas, as well as to support agricultural/forestry sectors for remaining productive in European areas affected by Xf outbreaks. *For more information about the BeXyl project: https://bexylproject.org/*

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The EFSA Organising committee: Francesco Amoretti, Alice Cantino, Alice Delbianco, Vanessa Descy, Filippo Positano, Giuseppe Stancanelli

The EFSA PLANTS social media team: Alexia Antoniou, Ewelina Czwienczek, Marina Elena Martino, Júlia López Mercadal, Sara Tramontini

The Pomilio Blumm team: Alessandra Del Vecchio, Carolina Vastola

The ICPP2023 Organising committee and EUROPA team