



# 2<sup>nd</sup> European conference on **Xylella fastidiosa** **2019**

HOW RESEARCH CAN SUPPORT SOLUTIONS

Ajaccio, 29-30 October 2019

**DRAFT**

**BOOK OF ABSTRACTS**



**Note:** For all abstracts only the affiliation of the presenter is listed. The abstracts have been edited and proofread in British English by Wiley. The book of abstracts will be published after the conference on *EFSA Supporting Publications* on Wiley Online Library. In case you wish to correct your abstract before publication, please write to [xylella\\_conference@efsa.europa.eu](mailto:xylella_conference@efsa.europa.eu) by 10<sup>th</sup> November 2019.

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## Introduction

### Second European conference on *Xylella fastidiosa*

The second scientific conference on ongoing research into *Xylella fastidiosa* is being held in Ajaccio, Corsica, from 29 to 30 October 2019.

A post-conference field visit organised by the OEC on 31 October will allow participants to become familiar with the Corsican landscape, crops and potential host plants for *X. fastidiosa* and its vectors in this environment.

The latest results from European research projects dealing with *X. fastidiosa* will be presented, in particular the final results from the Horizon 2020 [POnTE](#) project. The conference will also be an opportunity for participants to catch up on the latest advances and achievements of other national and transnational research activities that are ongoing worldwide and to discuss ways of strengthening coordination and synergies.

The conference is organised jointly by [EFSA](#)<sup>1</sup>; the [French National Institute for Agricultural Research](#) (INRA); the [French Agency for Food, Environmental and Occupational Health and Safety](#) (ANSES); the Office de l'Environnement de la Corse (OEC) through its department the [Conservatoire Botanique National de Corse](#); the EU-funded projects [POnTE](#), [XF-ACTORS](#), [CURE-XF](#) and [EuroXanth](#); and the [Euphresco](#) network for phytosanitary research coordination and funding.

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<sup>1</sup> [EFSA: second European conference on Xylella fastidiosa](#)

## Acknowledgments



The Scientific and Organising committees wish to thank all the organisations and individuals that contributed to the realisation of the second European conference on *Xylella fastidiosa*.

We are very grateful to EFSA for the overall organisation of the conference, and to CNBC OEC for offering the Centre de Congrès d'Ajaccio as venue for the conference as well as for kindly organising the field visit in collaboration with INRA.

We would also like to thank EFSA, EuroXanth COST Action and CURE XF for their financial support to the Young Researchers' Initiative.

Many thanks also to POnTe for offering the Welcome and networking cocktail and for supporting the participation of different stakeholders.

Last, but not least, we would like to thank all chairmen of the respective sessions and all the people involved in the organisation.

<b>Scientific committee</b>	
Claude Bragard	<i>Chair of the Scientific Committee of the second European conference on Xylella fastidiosa Chair of EFSA Plant Health Panel Earth&amp;Life Institute, UCLouvain (BE)</i>
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## Programme

### **2<sup>nd</sup> European conference on *Xylella fastidiosa*: how research can support solutions Ajaccio, 29<sup>th</sup> – 30<sup>th</sup> October 2019**

#### **Monday 28<sup>th</sup> October 2019**

18.00-21.00	<b>Registration and posters hanging</b> <i>Hall d'exposition Napoléon Bonaparte</i>
19.00-21.00	<b>Welcome and networking cocktail offered by POnTE project to all participants of the 2<sup>nd</sup> European conference on <i>Xylella fastidiosa</i></b> <i>Hall d'exposition Napoléon Bonaparte</i>
20.00-20.15	<b>Opening ceremony</b> <i>Hall d'exposition Napoléon Bonaparte</i>





2<sup>nd</sup> European  
conference on  
**Xylella  
fastidiosa**  
**2019**

## 2<sup>ND</sup> EUROPEAN CONFERENCE ON XYLELLA FASTIDIOSA: HOW RESEARCH CAN SUPPORT SOLUTIONS

Ajaccio 29<sup>th</sup> - 30<sup>th</sup> October 2019

### DAY 1 - Tuesday 29<sup>th</sup> October

07.30 - 09.00	Registrations and poster hanging	
09.00 - 10.30	Opening session	Auditorium Pascal Paoli
10.30 - 11.00	Coffee/Tea break	Restaurant Tino Rossi
11.00 - 13.00	Session 1: Biology and pathogenicity I	Auditorium Pascal Paoli
13.00 - 14.00	Lunch	Restaurant Tino Rossi
14.00 - 16.00	Session 2: Vectors I	Auditorium Pascal Paoli
	Session 3: Detection	Room Sampiero Corso
16.00 - 16.30	Coffee/Tea break	Restaurant Tino Rossi
16.30 - 18.30	Session 4: Ecology, Epidemiology and modelling	Auditorium Pascal Paoli
	Session 5: Vectors II	Room Sampiero Corso
18.45 - 21.00	Cocktail and Poster session	
		Hall d'exposition Napoléon Bonaparte

### DAY 2 - Wednesday 30<sup>th</sup> October

08.30 - 10.30	Session 6: Risk and impact assessment	Auditorium Pascal Paoli
10.30 - 11.00	Coffee/Tea break	Restaurant Tino Rossi
11.00 - 13.00	Session 7: Surveillance	Auditorium Pascal Paoli
	Session 8: Biology and pathogenicity II	Room Sampiero Corso
13.00 - 14.00	Lunch	Restaurant Tino Rossi
14.00 - 16.00	Session 9: Sustainable control measures	Auditorium Pascal Paoli
16.00 - 16.30	Coffee/Tea break	Restaurant Tino Rossi
16.30 - 18.30	Final session: What achievements and new research targets for control of <i>X. fastidiosa</i> ?	Auditorium Pascal Paoli

### DAY 3 - Thursday 31<sup>st</sup> October

08.00 - 16.00	Field visit
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**DAY 1 – Tuesday 29<sup>th</sup> October 2019**

07.30-09.00	<b>Registration and posters hanging</b> <i>Hall d'exposition Napoléon Bonaparte</i>	
<b>OPENING SESSION - Auditorium Pascal Paoli</b> <b>Chair: Claude Bragard</b> , Chair of EFSA Plant Health Panel		
09.00-09.10	Welcome to the conference	<b>Julien Paolini</b> University of Corsica Pasquale Paoli (FR)
09.10-09.20	Introduction to the conference	<b>Claude Bragard</b> Chair of EFSA Plant Health Panel
09.20-09.50	Advances in molecular and ecological studies for the control of <i>Xylella fastidiosa</i>	<b>Steven Lindow</b> University of California Berkeley (US)
09.50-10.00	Q&A	
10.00-10.20	Major results and challenges of the EU H2020 POnTE project on the control of <i>Xylella fastidiosa</i>	<b>Donato Boscia</b> Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR) (IT)
10.20-10.30	Q&A	
10.30-11.00	<b>Coffee/Tea break - Restaurant Tino Rossi</b>	
<b>SESSION 1: Biology and pathogenicity I – Auditorium Pascal Paoli</b> <b>Chair: Maria Saponari</b> , Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR) (IT)		
11.00-11.15	Understanding the potential origin and epidemiological consequences of the Spanish outbreaks caused by <i>Xylella fastidiosa</i> subspecies <i>multiplex</i>	<b>Blanca Landa</b> Institute of Sustainable Agriculture (IAS-CSIC) (ES)
11.15-11.20	Q&A	
11.20-11.35	Evolutionary history of <i>Xylella fastidiosa</i> based on comparative genomics	<b>Marie-Agnès Jacques</b> Institut National de la Recherche Agronomique (INRA) (FR)
11.35-11.40	Q&A	
11.40-11.55	Insights into differential responses of olive cultivars to <i>Xylella fastidiosa</i> infections	<b>Pasquale Saldarelli</b> Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR) (IT)
11.55-12.00	Q&A	
12.00-12.15	Extent and ecological significance of homologous recombination in <i>Xylella fastidiosa</i>	<b>Leonardo De La Fuente</b> Auburn University (US)
12.15-12.20	Q&A	
12.20-12.35	Can genome sequences tell us anything worthwhile about <i>Xylella fastidiosa</i> ecology?	<b>Rodrigo Almeida</b> University of California Berkeley (US)
12.35-12.40	Q&A	

12.40-13.00	Questions and discussion	
13.00-14.00	<b>Lunch - Restaurant Tino Rossi</b>	
<b>SESSION 2: Vectors I – Auditorium Pascal Paoli</b>		
<b>Chair: João R.S. Lopes</b> , Dept. Entomology and Acarology University of São Paulo/ESALQ (BR)		
14.00-14.15	Reduction in vector acquisition and transmission efficiency of <i>Xylella fastidiosa</i> subsp. <i>pauca</i> by blocking the afimbrial protein XadA2	<b>João R.S. Lopes</b> , Dept. Entomology and Acarology University of São Paulo/ESALQ (BR)
14.15-14.20	Q&A	
14.20-14.35	Reconstruction of the plant-vector trophic networks involved in the spread of <i>Xylella fastidiosa</i> through hybrid capture	<b>Jean-Yves Rasplus</b> Institut National de la Recherche Agronomique (INRA) (FR)
14.35-14.40	Q&A	
14.40-14.55	Mark-recapture experiments to estimate the dispersal capacity of <i>Philaenus spumarius</i>	<b>Anna Simonetto</b> University of Brescia (IT)
14.55-15.00	Q&A	
15.00-15.15	Phenology and host-plant association of spittlebugs in Mediterranean olive groves	<b>Domenico Bosco</b> University of Torino (IT)
15.15-15.20	Q&A	
15.20-15.35	Use of vibrations to manipulate the behaviour of the meadow spittlebug <i>Philaenus spumarius</i>	<b>Sabina Avosani</b> University of Trento (IT)
15.35-15.40	Q&A	
15.40-16.00	Questions & discussion	
16.00-16.30	<b>Coffee/Tea break - Restaurant Tino Rossi</b>	
<b>SESSION 3: Detection - Room Sampiero Corso</b>		
<b>Chair: Françoise Petter</b> , European and Mediterranean Plant Protection Organization (EPPO)		
14.00-14.15	Novel and high-throughput diagnostic procedures to detect <i>Xylella fastidiosa</i> in planta and vectors developed within POnTE project	<b>Françoise Poliakoff</b> Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES) (FR)
14.15-14.20	Q&A	
14.20-14.35	New tetraplex qPCR assays for simultaneous detection and identification of <i>Xylella fastidiosa</i> subspecies in plant tissues	<b>Enora Dupas</b> Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES) (FR) Institut National de la Recherche Agronomique (INRA) (FR)
14.35-14.40	Q&A	
14.40-14.55	Validation of real-time PCR assays for the specific detection of each <i>Xylella fastidiosa</i> sub-species	<b>Jennifer Cole</b> Fera Science Ltd (Fera) (UK)
14.55-15.00	Q&A	

15.00-15.15	A quick and efficient method for detection of <i>Xylella fastidiosa</i> in olive plants based on tissue-print	<b>Helvecio della Coletta-Filho</b> Campinas Agronomic Institute (IAC), APTA Citrus Sylvio Moreira Center (BR)
15.15-15.20	Q&A	
15.20-15.35	Optimization of sampling and testing procedures for detecting <i>Xylella fastidiosa</i> in large lots of plants for planting and nursery stocks	<b>Giuliana Loconsole</b> University of Bari Aldo Moro (IT)
15.35-15.40	Q&A	
15.40-16.00	<i>Questions &amp; discussion</i>	
16.00-16.30	<b>Coffee/Tea break - Restaurant Tino Rossi</b>	
<b>SESSION 4: Ecology, epidemiology and modelling - Auditorium Pascal Paoli</b> <b>Chair: Rodrigo Krugner, Agricultural Research Service (ARS, USDA) (US)</b>		
16.30-16.45	Temperature determines growth and biofilm formation of <i>Xylella fastidiosa</i> strains in vitro	<b>Juan A. Navas-Cortés</b> Institute for Sustainable Agriculture (IAS-CSIC) (ES)
16.45-16.50	Q&A	
16.50-17.05	Developing a spatial epidemiological model to estimate <i>Xylella fastidiosa</i> dispersal and spread	<b>Daniel Chapman</b> University of Stirling (UK)
17.05-17.10	Q&A	
17.10-17.25	An epidemiological model for the short-range spread of <i>Xylella fastidiosa</i> and the assessment of eradication management measures	<b>Gianni Gilioli</b> University of Brescia (IT)
17.25-17.30	Q&A	
17.30-17.45	Risk maps and risk-based surveillance for early detection of <i>Xylella fastidiosa</i> in continental France	<b>Davide Martinetti</b> Institut National de la Recherche Agronomique (INRA) (FR)
17.45-17.50	Q&A	
17.50-18.05	The spreading of <i>Xylella fastidiosa</i> subsp. <i>pauca</i> among the olive orchards of Southern Italy (Apulia)	<b>Enrico Bucci</b> Sbarro Health Research Organization (SHRO)-Temple University (US)
18.05-18.10	Q&A	
18.10-18.30	<i>Questions &amp; discussion</i>	
<b>SESSION 5: Vectors II – Room Sampiero Corso</b> <b>Chair: Michael Maixner, Federal Research Centre for Cultivated Plants, Julius Kühn Institute (JKI) (DE)</b>		
16.30-16.45	Insights into the transmission dynamics of <i>Xylella fastidiosa</i> by <i>Philaenus spumarius</i>	<b>Daniele Cornara</b> Institute of Sustainable Agriculture (IAS-CSIC) (ES)
16.45-16.50	Q&A	
16.50-17.05	A barcode database to identify the vectors of <i>Xylella fastidiosa</i> in Europe	<b>Jean-Claude Streito</b> Institut National de la Recherche Agronomique (INRA) (FR)
17.05-17.10	Q&A	

17.10-17.25	Detection, identification and surveillance of <i>Xylella fastidiosa</i> on vectors in France	<b>Amandine Cuntz</b> Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES) (FR)
17.25-17.30	Q&A	
17.30-17.45	Transmission characteristics of <i>Xylella fastidiosa</i> subsp. <i>pauca</i> (ST53) by <i>Philaenus spumarius</i> and <i>Cicadella viridis</i>	<b>Nicola Bodino</b> University of Torino (IT)
17.45-17.50	Q&A	
17.50-18.00	Flight behaviour of <i>Philaenus spumarius</i> , the main vector of <i>Xylella fastidiosa</i>	<b>Clara Lago</b> Institute of Sustainable Agriculture (IAS-CSIC) (ES)
18.00-18.10	Host plant affiliation of xylem-feeders within Central Europe	<b>Anna Markheiser</b> Julius Kühn Institute (JKI) (DE)
18.10-18.30	<i>Questions &amp; discussion</i>	
<b>18.45-21.00</b>	<b>Cocktail and Poster session - Hall d'exposition Napoléon Bonaparte</b>	
19.00-20.00	Presentation of posters with odd numbers	
20.00-21.00	Presentation of posters with even numbers	

**DAY 2 – 30<sup>th</sup> October 2019**

<b>SESSION 6: Risk and impact assessment – Auditorium Pascal Paoli</b>		
<b>Chair: Blanca Landa</b> , Higher Council for Scientific Research (CSIC) (ES)		
08.30-08.45	Monitoring the impact of <i>Xylella</i> on Apulia's olive orchards using Sentinel-2 satellite data and aerial photographs	<b>Pieter Beck</b> Joint Research Centre (JRC), European Commission (EC)
08.45-08.50	Q&A	
08.50-09.05	An updated assessment of the risks to plant health posed by <i>Xylella fastidiosa</i> in the EU territory	<b>Stephen Parnell</b> Salford University (UK)
09.05-09.10	Q&A	
09.10-09.25	Potential impact of <i>Xylella fastidiosa</i> subsp. <i>pauca</i> in European olives: a bio-economic analysis	<b>Kevin Schneider</b> Wageningen University & Research (NL)
09.25-09.30	Q&A	
09.30-09.45	Estimating the economic, social and environmental impacts of European priority pests: a joint project for EFSA and JRC and the case study of <i>Xylella fastidiosa</i>	<b>Berta Sánchez</b> Joint Research Centre, European Commission (JRC) and <b>Olaf Mosbach-Schulz</b> European Food Safety Authority (EFSA)
09.45-09.50	Q&A	
09.50-10.05	Living with <i>Xylella</i> : The dynamics of knowledges within <i>X. fastidiosa</i> "sociopathosystems" in Puglia and Corsica	<b>Christian Colella</b> University of Milano-Bicocca (IT)
10.05-10.10	Q&A	

10.10-10.30	Questions & discussion	
10.30-11.00	<b>Coffee/Tea break - Restaurant Tino Rossi</b>	
<b>SESSION 7: Surveillance – Auditorium Pascal Paoli</b>		
<b>Chair: Philippe Reignault</b> , Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES) (FR)		
11.00-11.15	Targeting surveillance for <i>Xylella fastidiosa</i> in Europe: an epidemiological basis	<b>Alexander Mastin</b> University of Salford (UK)
11.15-11.20	Q&A	
11.20-11.35	Spatio-temporal monitoring of <i>Xylella fastidiosa</i> in olive trees using radiative transfer models and Sentinel-2 images	<b>Alberto Hornero</b> Swansea University (UK)
11.35-11.40	Q&A	
11.40-11.55	A survey in Israel reveals the presence of <i>Xylella fastidiosa</i> in almond trees in the northern part of the country	<b>Ofir Bahar</b> Agricultural Research Organization (ARO) (IL)
	Q&A	
12.00-12.15	Optimization of the delimiting survey strategies for <i>Xylella fastidiosa</i> in the demarcated area in Alicante	<b>Elena Lázaro Hervás</b> Instituto Valenciano de Investigaciones Agrarias (IVIA) (ES)
12.15-12.20	Q&A	
12.20-12.35	EFSA tool kit for <i>Xylella fastidiosa</i> risk based surveillance in the EU Member States	<b>Sybren Vos</b> European Food Safety Authority (EFSA)
12.35-12.40	Q&A	
12.40-13.00	Questions & discussion	
13.00-14.00	<b>Lunch break - Restaurant Tino Rossi</b>	
<b>SESSION 8: Biology and pathogenicity II – Room Sampiero Corso</b>		
<b>Chair: Marie-Agnès Jacques</b> , Institut National de la Recherche Agronomique (INRA) (FR)		
11.00-11.15	Spatial distribution and genetic structure of <i>Xylella fastidiosa</i> subsp. <i>pauca</i> in Olive trees in Southeast Brazil	<b>Nagela Safady</b> Centro de Citricultura Sylvio Moreira (CCSM-IAC) (BR) Federal University of São Carlos (UFSCar) (BR)
11.15-11.20	Q&A	
11.20-11.35	Cultivar susceptibility and temperature-dependent recovery of <i>Xylella fastidiosa</i> -infected grapevines	<b>Lindsey Burbank</b> Agricultural Research Service (USDA) (US)
11.35-11.40	Q&A	
11.40-11.55	Phenotypic characterization of two Spanish strains of <i>Xylella fastidiosa</i> subsp. <i>multiplex</i> ST6 differing in plasmid content	<b>Miguel Román-Écija</b> Institute for Sustainable Agriculture (IAS-CSIC) (ES)
11.55-12.00	Q&A	
12.00-12.15	Ancestral state reconstructions of <i>Xylella fastidiosa</i> -host plant relationships	<b>Alexandra Kahn</b> University of California Berkeley (US)
12.15-12.20	Q&A	

12.20-12.35	Rethinking the <i>Xylella fastidiosa</i> scenario in the Balearic Islands: what epidemiological, phylogenetic and dendrochronological data tell us	<b>Eduardo Morelejo</b> Empresa de Transformación Agraria (Tragsa) (ES)
12.35-12.40	Q&A	
12.40-13.00	<i>Questions &amp; discussion</i>	
13.00-14.00	<b>Lunch break - Restaurant Tino Rossi</b>	
<b>SESSION 9: Sustainable control measures – Auditorium Pascal Paoli</b>		
<b>Chair: Rodrigo Almeida, University of California Berkeley (US)</b>		
14.00-14.15	The VSPP, a voluntary certification programme to produce healthier plants for planting in the EU	<b>Camille Picard</b> European and Mediterranean Plant Protection Organization (EPPO)
14.15-14.20	Q&A	
14.20-14.35	N-acetyl-cysteine for controlling <i>Xylella fastidiosa</i> in citrus and olive: understanding the differences to improve management	<b>Alessandra de Souza</b> Centro de Citricultura Sylvio Moreira (CCSM-IAC) (BR)
14.35-14.40	Q&A	
14.40-14.55	Further acquisition on the response of a large number of olive cultivars to infections caused by <i>Xylella fastidiosa</i> subsp. <i>pauca</i> , ST53	<b>Donato Boscia</b> Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR) (IT)
14.55-15.00	Q&A	
15.00-15.15	Strategies for reducing vector populations and transmission of <i>Xylella fastidiosa</i> in olive groves	<b>Crescenza Dongiovanni</b> Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile Caramia" (CRSFA) (IT)
15.15-15.20	Q&A	
15.20-15.30	Understanding the olive microbiome of susceptible and resistant cultivars for sustainable biocontrol	<b>Paula Baptista</b> Polytechnic Institute of Bragança (IPB) (PT)
15.30-15.40	Assessment of <i>Paraburkholderia phytotfirmans</i> PsJN biocontrol potential against <i>Xylella fastidiosa</i> 'De Donno' strain in olive	<b>Massimiliano Morelli</b> Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR) (IT)
15.40-16.00	<i>Questions &amp; discussion</i>	
16.00-16.30	<b>Coffee/Tea break - Restaurant Tino Rossi</b>	

<b>FINAL SESSION:</b>		
<b>What achievements and new research targets for control of <i>X. fastidiosa</i>?</b>		
<b>Discussion with stakeholders and conference conclusions</b>		
<i>Auditorium Pascal Paoli</i>		
<b>Chair: Giuseppe Stancanelli, European Food Safety Authority (EFSA)</b>		
16.30-16.45	European Farmers' and Agri-Cooperatives' point of view on next research priorities for control of <i>X. fastidiosa</i>	<b>Luc Peeters</b> Chair of Phytosanitary Questions working group, COPA-COGECA
16.45-17.05	<i>Discussion with all the conference Chairs</i>	
17.05-17.20	European Nurseries' point of view on next research priorities for control of <i>X. fastidiosa</i>	<b>Josep M. Pagès</b> European Nurserystock Association (ENA)
17.20-17.40	<i>Discussion with all the conference Chairs</i>	
17.40-17.55	Risk managers' point of view on next research priorities for control of <i>X. fastidiosa</i>	<b>Pasquale Di Rubbo</b> European Commission, DG SANTE
17.55-18.15	<i>Discussion with all the conference Chairs</i>	
18.15-18.30	Final conclusions	<b>Claude Bragard</b> Chair of EFSA Plant Health Panel

**DAY 3 – 31<sup>st</sup> October 2019**

08.00-16.00	<b>Field visit organised by CNBC-OEC and INRA</b>
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# Oral Presentations

## Opening Session

### Advances in molecular and ecological studies for the control of *Xylella fastidiosa*

Lindow S\*

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**Abstract:** *Xylella fastidiosa* has a complex lifestyle since it also colonises the mouthparts of xylem-feeding insect vectors. Expression of about 10% of the genome is influenced by accumulation of C14 and C16 cis 2-unsaturated fatty acids (diffusible signalling factors, DSF) encoded by *rpfF* that serve as quorum-sensing signal molecules. DSF accumulation increases the adhesiveness and biofilm formation in *X. fastidiosa* required for insect colonisation and transmission but reduces colonisation of xylem vessels. Control of Pierce's disease of grape in both greenhouse and field trials has been achieved by a process of 'pathogen confusion' whereby expression of *rpfF* in plants confers constitutive production of DSF that increases the adhesiveness and reduces the active movement of *X. fastidiosa* in the plant. The endophytic bacterium *Paraburkholderia phytofirmans* strain PsJN has also been very effective in reducing Pierce's disease. It grew to 10<sup>6</sup> cells/g and moved over 1 metre within 4 weeks of inoculation of vines. Few viable cells of *X. fastidiosa* were recovered when it was co-inoculated with strain PsJN and disease severity was always greatly reduced. Large populations of strain PsJN could be established in both leaf lamina and petioles by topical application of cell suspensions in 0.2% of an organo-silicon surfactant conferring low surface tension; such treatments were as effective as direct puncture inoculations of this biocontrol strain in reducing disease severity. Inoculation of strain PsJN into plants at the same time as or even 4 weeks after the pathogen resulted in large reductions in disease severity; much less disease control was conferred by inoculation 4 weeks prior to that of the pathogen. The expression of grapevine PR1 and ETR1 was substantially higher in plants inoculated with both *X. fastidiosa* and strain PsJN compared to that in plants inoculated only with the pathogen or strain PsJN, suggesting that this biological control agent primes innate disease resistance pathways in plants.

### Major results and challenges of the EU H2020 POnTE project on the control of *Xylella fastidiosa*

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** 'Pest Organisms Threatening Europe' (POnTE) is a four-year H2020 Project (grant agreement number 635646), started in November 2015, targeting emerging pests that threaten EU agriculture and forestry to develop knowledge and tools to minimise their impact and the risk of introduction in pest-free areas. The bacteria *Xylella fastidiosa* and *Candidatus Liberibacter solanacearum* and their vectors, as well as the phytopathogenic fungi causing major diseases in forestry and landscape trees, i.e. *Phytophthora* spp. and *Hymenoscyphus* spp., were studied by a multidisciplinary team representing more than 20 Partners.

The most relevant part of the project workplan was dedicated to studying the biological, genetic and epidemiological aspects of the bacterium *X. fastidiosa* spreading in olives in southern Italy.

The bacterium, its vectors and the host response has been explored using innovative approaches and the knowledge gathered has significantly improved the current methods for diseases surveillance and prevention. Whereas, the preliminary results of the studies on the spittlebugs, olive microbiome and mechanisms of resistance in olive cultivars opened up new opportunities for the implementation of future applied research programmes on sustainable control strategies. The research programme has been supported by intense communication and dissemination actions, promoting research networking, harmonisation of the surveillance strategies and transfer of knowledge to stakeholders. Several scientific evidences collected in the framework of the project have been used to support and update the pest risk assessment and to implement the legislative measures enforced at EU and national level.

The most significant achievements of the project will be briefly illustrated.

## Session 1: Biology and pathogenicity I

### Understanding the potential origin and epidemiological consequences of the Spanish outbreaks caused by *Xylella fastidiosa* subspecies *multiplex*

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** Outbreaks in Europe associated with *Xylella fastidiosa* (Xf) subspecies *multiplex* are the most frequent, accounting for the larger number of susceptible plant species (more than 70%), with some host overlap among the different regions. Diverse Sequence Types (ST) have been detected across distinct geographical regions: ST6, ST7 and ST79 in Corsica and PACA region (France), ST7 and ST81 in the Balearic Islands (Spain), ST6 in the province of Alicante and Madrid (mainland Spain), ST7 in the Douro Littoral region (Portugal), and ST87 in the region of Tuscany (northern Italy). In recent years, genetic analysis and pathogenicity tests have provided evidence of biological, ecological and host range diversity among strains of the same subspecies and STs. Draft genomes of 12 Spanish isolates of Xf subsp. *multiplex* (ST6 and ST81) were used for comparative genomic studies with currently available genomes of the same subspecies from France and Italy. Phylogenetic analysis based on core genomes, accessory genomes and single nucleotide polymorphisms indicate that: (i) European outbreaks associated with strains of Xf subsp. *multiplex* most likely result from distinct independent introductions; (ii) ST6-strains recovered from Spain and France, although sharing the same ST, fell in distinct phylogenetic subgroups; (iii) ST81 strains from the Balearic Islands and ST6 strains from Alicante differentiated in distinct phylogenetic groups (i.e. ST81 isolates are closer to ST6 isolates from California and France than ST6 isolates from Alicante, which in turn are closer to ST7 isolates from the USA and France); (iv) a low number of SNPs are detected among the strains recovered in Alicante, suggesting a recent introduction in the area. Additionally, whereas some recombination events were found among ST81 isolates from Mallorca and ST6 isolates from France with isolates of Xf subsp. *fastidiosa* ST1 from Mallorca, no evidence of recombination among ST6 isolates from Alicante with other European Xf isolates was found. Pathogenicity tests on the three main Spanish olive cultivars are being conducted with isolates belonging to ST6 and ST81 and compared to Xf subsp. *pauca* ST53 from Italy and ST80 from Ibiza. This work highlights that although the use of MLST approach is a powerful tool for resolving genetic relationships among isolates, the exploration of the whole genomes brings to more comprehensive information that in the future may help to retrieve more robust correlations with the biology and host range of the sequenced isolates. Furthermore, since current European regulation of Xf is based on the

subspecies present in each outbreak, these results combined with further pathogenicity tests on main crops may help to establish management and regulation policy standards for the affected areas in Europe.

### *Acknowledgements*

This work has received funding from projects 727987 XF-ACTORS (EU-H2020), E-RTA2017-00004-C06-02 from AEI-INIA Spain and FEDER, and the Spanish Olive Oil Interprofessional.

## **Evolutionary history of *Xylella fastidiosa* based on comparative genomics**

Jacques M-A\*, Briand M, Denancé N, Durand K, Dupas E, Rieux A, Cesbron S

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** Once thought to be restricted to the Americas, the plant-associated bacterium *Xylella fastidiosa* has been blooming since 2013 in Europe and Asia. This bacterium infects a large host range and is responsible for plant diseases with great socioeconomic consequences. Genetically diverse, this species is divided into subspecies but genetic traits governing this classification are poorly understood. We used comparative genomics of a set of nearly 50 genome sequences to gain better knowledge on genetic traits associated to lineages and gene fluxes among lineages. We developed a software, SkIf (Specific k-mers Identification), to mine genome sequences and identify signatures of groups of interest. Genome sequences were also used to gain knowledge in the evolutionary history of the subspecies *multiplex*. Altogether, we provide important resources and knowledge to optimise the strategies attempted to limit the pathogen dissemination in novel areas.

### *Bibliography*

Denancé N, M Briand, R Gaborieau, S Gaillard, M-A Jacques. 2019. Identification of genetic relationships and subspecies signatures in *Xylella fastidiosa*. BMC Genomics 20:239.

## **Insights into differential responses of olive cultivars to *Xylella fastidiosa* infections**

Abou Kubaa R, Giampetruzzi A, Altamura G, Zicca S, Boscia D, Saponari M, Saldarelli P\*

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** *Xylella fastidiosa* strain De Donno causes severe symptoms of desiccation on the susceptible cultivars Ogliarola salentina and Cellina di Nardò. In the *Xylella*-ravaged olive groves, survivor plants of cv Leccino have been identified and monitored since the beginning of epidemic spread of *X. fastidiosa* in Apulia (southern Italy). Studies in field-grown plants (Giampetruzzi et al., 2016) suggest that the resistance of these two cultivars relies on two pillars: a lower bacteria population size compared with that of susceptible cultivars and, limited to Leccino, a differential gene expression response that involves Leucine Rich Receptor-like kinases (LRR-RLKs). Successive studies with artificially infected olives under controlled conditions, showed that the same host responses occurred when plants of the cvs Cellina di Nardò and Leccino were inoculated with the strain CO33, taxonomically related to subsp. *sandyi*. Consistently with the previous data, transcripts of two LRR-RLKs, orthologous to At1g35710 and At4g08850, which are reported to regulate cell wall damage response in *Arabidopsis thaliana* (Van der Does et al., 2017), were found overexpressed in Leccino. Moreover, quantitative PCR assays targeting the At1g35710 olive orthologous gene showed an increased expression in different olive cultivars artificially inoculated under controlled conditions. Besides these molecular studies, the

bacterial population sizes were estimated in different tissues of the infected plants: leaves, young ( $\varnothing \leq 5\text{mm}$ ) and hardwood cuttings ( $\varnothing \geq 5\text{mm} - \leq 1\text{cm}$ ), and in tissues collected from scions of different cultivars grafted onto the same rootstock. From these tests, further evidence on the resistance of Leccino and FS17 were collected, with tissues of these cvs harbouring lower bacterial titer than Ogliarola salentina (this was particularly evident in the leaves of the cv Leccino) and not showing typical and severe desiccation phenomena, even when branches of these cvs were co-grafted on the same rootstocks with scions of Ogliarola salentina showing severe dieback. Further data on these double grafted olives and genetic achievements will be presented.

#### *Acknowledgement*

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 635646 'Pest Organisms Threatening Europe POnTE' and grant agreement N. 727987 'Xylella fastidiosa Active Containment Through a Multidisciplinary-Oriented Research Strategy XF-ACTORS'.

### **Extent and ecological significance of homologous recombination in *Xylella fastidiosa***

Potnis N, Merfa MV, Kandel PP, Cobine PA, De La Fuente L\*

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**Abstract:** *Xylella fastidiosa* is a naturally competent bacteria; therefore it is able to internalise exogenous homologous fragments of DNA and incorporate them into its genome. In the past we have studied conditions that are conducive for natural competence in the laboratory. For this study we aimed to determine the extent and explore the ecological significance of homologous recombination (HR) in the genomes of recombinants experimentally generated by natural competence in the laboratory and wild-type isolates. Both set of strains displayed widespread HR and similar average size of recombined fragments consisting of random events (2–10 kb) of inter- and intra-subspecific recombination. Significantly higher proportion and greater lengths (>10 kb, maximum 31.5 kb) of recombined fragments were observed in subsp. morus and in strains isolated in Europe from intercepted coffee plants shipped from the Americas. Such highly recombinant strains pose a serious risk of emergence of novel variants as genetically distinct and formerly geographically isolated genotypes are brought in close proximity by global trade. Recently recombined regions in wild-type strains included genes involved in regulation and signalling, host colonisation, nutrient acquisition and host defense evasion; all fundamental traits for *X. fastidiosa* ecology. Some of the identified recombined genes have been characterised in the past and were shown to be important for infection, while others have not been studied in *X. fastidiosa*. Identification of four recombinant loci shared between wild-type and experimentally generated recombinants suggest potential hotspots of recombination in this naturally competent pathogen. These findings provide insights into evolutionary forces possibly affecting the adaptive potential to colonise the host environments of *X. fastidiosa*.

### **Can genome sequences tell us anything worthwhile about *Xylella fastidiosa* ecology?**

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**Abstract:** While the benefits of using *Xylella fastidiosa* whole-genome sequences for academic research are rather obvious, it remains to be shown that these data provide tangible information of immediate relevance to affected stakeholders, disease managers, and policy makers. More specifically, the question is if whole-genome sequences provide additional and reliable information when compared to other approaches such as multi-locus sequence typing and biological assays, for example. Using a data set of approximately 350 genome sequences, we have been asking questions on pathogen dispersal as well as what

happens after introductions into new environments. We will provide examples and posit questions on the relative value of genome sequencing data to study and monitor *X. fastidiosa* ecology.

## Session 2: Vectors I

### Reduction in vector acquisition and transmission efficiency of *Xylella fastidiosa* subsp. *pauca* by blocking the afimbrial protein XadA2

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**Abstract:** The success of *Xylella fastidiosa* transmission by sharpshooters (Hemiptera: Cicadellidae: Cicadellinae) depends, among other factors, on the ability of the bacterium to attach and form a biofilm on the insect foregut surface. It is known that the interaction of *X. fastidiosa* with the digestive tract of the insect vector is mediated by bacterial membrane adhesion proteins. Measures aimed at disrupting this interaction can result in efficient transmission-blocking tactics. The objective of this work was to study the interference in the process of adhesion of a citrus strain (9a5c) *X. fastidiosa* subsp. *pauca* in the insect vector, using the afimbrial adhesin XadA2 as a blocking target. An *in vitro* acquisition system was used to submit 60 adults of the sharpshooter vector, *Macugonalia leucomelas*, to the following treatments: I) artificial diet (L-glutamine, L-asparagine and sodium citrate) and bacterial cells (108 CFU/mL); II) Artificial diet, bacterial cells (108 CFU/mL) and anti-XadA2 antibody (2:1,000); and III) artificial diet without bacterial cells (negative control). After a 6-h acquisition access period on these diets the insects were placed on a non-host plant of *X. fastidiosa* (*Vernonia condensata*) for a 24-h gut-washing period and then transferred to a susceptible host plant (*Catharanthus roseus*) for a 72-h inoculation access period (IAP). The sharpshooters were evaluated for *X. fastidiosa* acquisition by qPCR right after the IAP, and 60 days later the inoculated *C. roseus* plants were assessed by standard PCR for detecting bacterial transmission. There was a drastic reduction in acquisition rate from the diet containing anti-XadA2 (18.4%) compared to the diet without this antibody (56.3%). The transmission rate of *X. fastidiosa* to *C. roseus* by individuals fed on bacterial suspensions containing anti-xadA2 (3.4%) was about three times lower than that of individuals fed on bacterial suspensions without it (10.7%). These results show that XadA2 can be used as a target to block *X. fastidiosa* subsp. *pauca* adhesion to the foregut of the vector, resulting in reduction of acquisition and transmission efficiency.

### Reconstruction of the plant–vector trophic networks involved in the spread of *Xylella fastidiosa* through hybrid capture

Rasplus J-Y\*, Gonzalez A-A, Chartois M, Nidelet S, Quiquerez I, Mesmin X, Farigoule P, Thuillier J-M, Streito J-C, Bosco D, Fried G, Hugot L, Casabianca F, Santoni S, Cruaud A

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**Abstract:** To infer plant–vector trophic networks and assess their impact on the spread of *Xylella fastidiosa* (Xf), we must answer three questions: which insects feed on which plant(s)? Which insects/plants are carrying Xf? Which strain(s) of Xf are we dealing with? Although these questions appear basic, they are technically challenging.

We demonstrated that current methods (qPCR, PCR) are not sensitive enough to detect Xf in vectors when the number of bacteria is lower than ca 250 and developed a nested PCR approach for the typing of Xf in insects. We used this technique to assess the prevalence of Xf in wild population of *Philaenus spumarius* throughout Corsica and managed, for the

first time, to sequence from a vector the seven housekeeping loci that are used for Xf typing in plants.

However, this method is not sensitive enough to i) sequence all loci when the number of bacteria lower than 50 and ii) detect the few plant cells ingested by vectors. Thus, we developed a new approach based on the target enrichment of gene regions through hybrid capture by RNA probes. Probes were designed i) from the 7 loci of the MLST scheme and ii) from ca 30K sequences of *rbcl*, the most represented marker in international databases to target European plant species.

We recently succeeded in isolating the DNA of *Cistus monspeliensis*, the preferred feeding plant of *P. spumarius* in Corsica, from specimens sampled in the field when pollen was absent. Likewise, we identified *Vitis* and *Quercus* DNA from American vectors sampled in California. We will present the results of a larger experiment still in progress.

Our results show that the capture of Xf genes (and flanking regions) and *rbcl* from insects is effective, which opens up new avenues for the reconstruction of networks of interaction for vector-borne plant diseases.

### **Mark-Recapture Experiments to estimate the dispersal capacity of *Philaenus spumarius***

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** The spread of the vectors is a key point in understanding the epidemiology of *Xylella fastidiosa* and in assessing vector control strategies. In Europe, the transmission of the bacterium is mainly due to spittlebugs. In particular, in the Apulia region (Italy) *Philaenus spumarius* has been proved to play the major role in transmitting *X. fastidiosa* subspecies *pauca*. Despite its importance, little information is available on the dispersal capacity of *P. spumarius*. To fill this knowledge gap mark-release-recapture experiments on *P. spumarius* adults were carried out in two agroecosystems: an olive grove and in a grass meadow, in the Apulia and the Piedmont regions (Italy), respectively. Dispersal capabilities of the vector were analysed in experiments performed from May to October in 2016 and 2017. Adults of *P. spumarius* of both sexes were captured in natural grassland habitats, marked with an aqueous solution of albumin and then released at a single point in the centre of the experimental area. The dispersal capacity was described estimating the probability density function describing the distribution of the end locations of insects relative to the source point (i.e. the dispersal kernel). Under the hypothesis of a random walk and applying a Gaussian kernel, diffusion rates in the two agroecosystems were estimated. Results showed a high variability in the estimated daily median distance from the release point, ranging from 19 to 51 metres. Considering that marked insects could disperse over an area wider than the experimental field, a correction for the truncated sampling bias has been included into the dispersal kernel, leading to a significant increase in the estimated daily median distances.

### **Phenology and host-plant association of spittlebugs in Mediterranean olive groves**

Bodino N, Cavalieri V, Dongiovanni C, Plazio E, Saladini MA, Volani S, Simonetto A, Fumarola G, Di Carolo M, Porcelli F, Gilioli G, Bosco D\*

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** Phenology and ecology of *Philaenus spumarius* and other spittlebug species were investigated during regular field surveys in 2016–2018 in four olive orchards located in coastal and inland areas of Apulia and Liguria regions of Italy, within the frame of an EFSA-funded project. Nymphal population in the herbaceous cover was estimated using quadrat samplings. Adults were collected by sweeping net on three different vegetational components: herbaceous cover, olive canopy and wild woody plants. Although the nymphs were polyphagous, they showed a strong host-preference for herbaceous plants of the Asteraceae and Fabaceae families in both the Liguria and Apulia regions of Italy: 72–88% of the total nymphs were indeed associated with these plant families. Nymphs of *Aphrophora* showed a similar host-preference, while those of *Neophilaenus* were strongly associated with Poaceae (85–100% of the nymphs were found on gramineous plants). *Aphrophora alni* and *N. campestris* showed a very low population density compared to *P. spumarius*. The average nymph population density of *P. spumarius* varied from 13 to 30 individuals/m<sup>2</sup> in Liguria according to the olive grove and the year, and from 5 to 19 individuals/m<sup>2</sup> in Apulia. Phenological data based on physiological time revealed that in Liguria the peak of abundance of *P. spumarius* nymph population was between 150 and 210 degree day (DD) while in Apulia the same peak was between 100 and 270 DD. This difference among locations could be explained by a non-linear component in the temperature-dependent development rate function of *P. spumarius*. The phenological pattern in the two regions is more similar if referred to chronological time. In fact, nymphs developed in Liguria between early March and end of May, and in Apulia between the end of February and mid-May. Field data are integrated with mesocosm and microcosm observations on the phenology and biology of *P. spumarius*.

## Use of vibrations to manipulate the behaviour of the meadow spittlebug *Philaenus spumarius*

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** Sexually mature adults of the meadow spittlebug, *Philaenus spumarius* (Linn.), exchange vibrational signals through the host plants to communicate and achieve mating. Novel pest control strategies involve the manipulation of the sexual behaviour of the insect by means of species-specific mechanical stimuli transmitted to plants. Playback trials with mini-shakers were conducted to evaluate if the transmission of pre-recorded *P. spumarius* vibrational signals to a plant of *Helianthus annuus* could affect the behaviour of the insect and to evaluate the potential use of vibrations for management practices against this pest. In all the trials, vibrational signals emitted by the specimens were recorded with laser vibrometer. In all the trials, vibrational signals emitted by the specimens were recorded with laser vibrometer. *P. spumarius* males and females were tested with playback of two types of male calling signal, which are spontaneously emitted by males since their emergence in spring and differ for the presence/absence of pulses within them. The playbacks were played in June-July (n=30) and in September-October (n=20) to evaluate whether the role of the signals could depend on the time of the season. In August and September, pairs consisting of a female and a male were released on different leaves of a *H. annuus* plant and stimulated either with the playback of pre-recorded signals such as the male rivalry signal (n=20) or the FRjS (n=20) or with a broadband noise (n=20) to disrupt the pair formation process. Results of all the playback trials are presented as well as a fine description of the mating behaviour of the meadow spittlebug. Insights on the

potential future development of more sustainable control strategies against this pest are given

## Session 3: Detection

### **Novel and high-throughput diagnostic procedures to detect *Xylella fastidiosa* in planta and vectors developed within POnTE project**

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** The research activities of the H2020 project POnTE partners explored serological and molecular methods to detect *Xylella fastidiosa* (Xf). Their efforts were devoted to optimize, standardize the procedures for sample preparation, purification of DNA, which are critical due to the presence of inhibitors in many host plants. Laboratories implemented internal comparative studies for accuracy, specificity, sensitivity and detection limit evaluation. As long with interlaboratories comparison tests, they permitted to assess performance criteria of the diagnostic methods. They allow to provide new set of data crucial to the revision of the EPPO Protocol (PM 7/24 (3)) and official methods. Direct sampling in the fields based on prints on cards/membrane procedures for serological tests with FTA-ELISA system as well as DTBIA are good alternatives to the standard sample extraction for ELISA. The Harper et al., (2010) protocol combined with CTAB or DNA extraction kit is still the most accurate method on almond, olive and other host DNA extracts. Quick biomolecular tests usable in the fields overcoming the preparation of sap and DNA extraction, such as olive prints on Whatman followed by qPCR, Lamp (Enbiotech kit) and RPA (Agdia kit) used with portable device are promising in terms of performance criteria but there is still a lack of experience. The direct nucleic acid hybridisation on nitrocellulose strip (lateral flow) is also an alternative that provided an optimisation to avoid amplicon contamination being carried out. Detection of Xf in heads of *Philaenus spumarium* in pools of 5 to 15 insects is reliable as performance criteria are not altered compared with the analysis of individual heads of insect using Taqman PCR Harper et al., (2010) in Duplex with IOOS et al., (2009) whatever DNA extraction used. Rapid protocols for subspecies assignation (single nucleotide primer extension – SnuPE) and HMR combined with end-point PCR have shown promising results: SNUPE method using a multiplex amplification of *gyrB* gene to differentiate all subspecies and genotypes within Xf subsp. *pauca* and a rapid and reliable protocol of HRM-analysis based on new set of primers in *leuA* and *gyrB* gene are on evaluation.

### **New tetraplex qPCR assays for simultaneous detection and identification of *Xylella fastidiosa* subspecies in plant tissues**

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** *Xylella fastidiosa* (Xf) is an insect-borne bacterium confined to the xylem vessels of plants. This pathogen has a broad host range estimated to more than 560 plant species. Five subspecies of the pathogen with different but overlapping host ranges have been



described, but only three are widely accepted. Detected in Europe since 2013, the management and regulation of its outbreaks in Europe depend on the subspecies. It is of major interest to identify it as accurately and as early as possible after infection. To improve Xf detection, three tetraplex qPCR tests were developed to identify the subspecies directly from plant material in a single reaction. All tests included primers designed to be specific of Xf species and different sets of primers targeting three subspecies. We designed primers and probes using SkIf, a bioinformatics tool, based on k-mers to detect specific signatures of the species and subspecies from a dataset of 47 genome sequences of Xf. We tested the qPCR assays on 39 target and 30 non-target strains, on samples of 13 different plant species spiked with Xf strains of different subspecies, and on naturally infected host plants samples. The primers and probes designed in this study were *in silico* as well as *in vitro* species- and subspecies-specific. On DNA, the sensitivity of single assays was equal or slightly better than the reference protocol, depending on the primers; and the tetraplex assays had the same sensitivity than the reference protocol. Tetraplex assays allow Xf detection up to 10<sup>3</sup> cells mL<sup>-1</sup> in all tested matrices. On naturally infected samples, the tetraplex qPCR tests allowed subspecies to be identified at levels where MLST failed. Moreover, mixed infections of two to three subspecies could be detected in the same sample with the tetraplex assays. These qPCR tests are modular tools reliable and efficient for differentiating Xf subspecies directly in plant samples.

### **Validation of real-time PCR assays for the specific detection of each *Xylella fastidiosa* subspecies**

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**Abstract:** Reliable identification to *Xylella fastidiosa* subspecies level requires time-consuming DNA sequencing of at least 7 genes using a multilocus sequence typing method. We developed rapid, targeted real-time PCR assays for the identification of each of the subspecies of *X. fastidiosa* direct from plant material. Diagnostic markers were identified using a comparative genomics approach to allow fine differentiation of the very closely related subspecies. Targeted assay design was then used to develop highly sub-specific diagnostic real-time PCR assays. Five assays were designed to allow detection of the described subspecies (*X. fastidiosa* subsp. *fastidiosa* and *multiplex*) and the proposed subspecies (*X. fastidiosa* subsp. *pauca*, *sandyi* and *morus*). The new assays are specific to the target subspecies, are as sensitive as the *X. fastidiosa* assay (Harper et al. 2010; erratum 2013), and have been fully validated in line with the EPPO standard PM7/98. Specificity was tested using non-target bacteria, healthy plants, and *X. fastidiosa*-infected material. Performance characteristics were compared with a widely used 'universal' *X. fastidiosa* assay (Harper et al. 2010; erratum 2013). The developed suite of assays allows the rapid determination of the *Xylella fastidiosa* subspecies present in unknown samples, resulting in a substantial reduction in the turnaround time for identification to the subspecies level compared with all existing diagnostic methods.

### **A quick and efficient method for detection of *X. fastidiosa* in olive plants based on tissue-print**

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**Abstract:** The commercial olive crop in southeast Brazil is relatively young, with plants no more than 20 years old. The olive orchards are located in a chain of mountains with altitude ranging from 800 to 1900 m, 1565 mm annual rainfall, and an average temperature of 19.4 °C, which results in biotic and abiotic disorders easily confounded with the olive quick decline syndrome (OQDS) symptoms associated with *X. fastidiosa* (Xf). The geographic localisation of orchards also makes it difficult for the shipment of samples (leaves) under conditions for diagnosis of Xf in the lab. Aiming to help the growers on identification of

OQDS symptoms in suspicious plants by the correlation with positive diagnosis of Xf, we are optimising the serologic-based tissue-print (T-P) protocol on nitrocellulose membrane for detection of the pathogen. Polyclonal antibodies were produced in rabbits using whole cells of a mixture of Xf subsp. *pauca* strains. Twigs and roots with 3 to 6 mm diameters of plants with OQDS-like symptoms were squeezed and the sap blotted onto membrane. The result of the detection was visualised directly on the membrane through colour reaction peroxidase-biotin-conjugated antibody. The validation of the method was checked by qPCR and PCR. Of total samples with OQDS-like symptoms, in 90% of the cases, the presence of Xf was confirmed by T-P, in 88% by q-PCR, and in only 33% by standard PCR. Xf was confirmed in 100% of the suspicious plants taking together both T-P and qPCR results. No infection of Xf was observed in healthy (asymptomatic) plants by the three tested methodologies. In conclusion, the T-P protocol was an efficient, cheaper and quicker methodology for Xf detection in plants with OQDS-like symptoms. Nitrocellulose membranes have been provided to the growers, which make the print of suspicious samples and later mail them to the lab. Interesting results have been obtained using this strategy. Support: Horizon 2020 (XF Actors project number 727987) and FAPESP (São Paulo Research Foundation – project number 2016/02176-7).

### **Optimisation of sampling and testing procedures for detecting *Xylella fastidiosa* in large lots of plant for planting and nursery stocks**

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** Inspections and diagnostic tests for *Xylella fastidiosa* are mandatory on consignments/place of productions for the most susceptible species listed in the EU Decision 2017/2352, as well as for the movement of 'specified plants' produced in nurseries located in the so-called demarcated areas. Although inspections and sampling are carried out in accordance with the ISPM31 standards, at laboratory level such requirements imply the manipulation of a large amount of materials (leaves/shoots/cuttings). Attempts have been made to experimentally optimise a protocol suitable for the concentration of the bacterial cells starting from large volume of plant homogenate, prior to being tested with the protocols commonly adopted for detecting the presence of *X. fastidiosa* in plants. Diagnostic sensitivity of serological and molecular tests was assessed by using infected plant materials (*X. fastidiosa* subspecies *pauca*, ST53) spiked at different ratio with non-infected plant materials. Portions (single leaves or pieces of stems) of infected plants were individually tested to define the distribution of the bacterium in a given infected symptomatic/asymptomatic plant. Briefly, petioles and/or stem portions recovered from infected plants of *Polygala myrtifolia*, *Nerium oleander*, *Olea europaea*, *Lavandula stoechas*, and scraped xylem tissue from infected cuttings of *Prunus avium* were pooled at different ratio with healthy materials up to 40 g/sample. Indeed, tests included plantlets from the species of the Brassicaceae and Solanaceae families (non-host plants of the ST53 strains) upon spiking the pooled samples with stem portions of infected periwinkle. After grinding the pooled samples, plant homogenates were centrifugated and the recovered pellets resuspended in the appropriate extraction buffer and processed using the standard serological or molecular protocols. The data gathered for sampling (minimum number of portions/aliquot) and for testing (maximum size of the pool) provide useful guidance when processing a large number of samples, satisfying sample size requirements while keeping the diagnostic tests technically and economically affordable.

*Acknowledgement*

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 727987 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS'.

## Session 4: Ecology, epidemiology and modelling

### Temperature determines growth and biofilm formation of *Xylella fastidiosa* strains *in vitro*

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** *Xylella fastidiosa* (Xf) is classified as a mesophilic bacterium particularly well adapted to areas with mild winters. The effects of temperature on Xf development have been addressed only for Xf subsp. *fastidiosa* strains. In this work, we have explored the effects of temperature on growth and biofilm formation *in vitro* for Xf strains representative of Xf subspecies prevalent in the European outbreaks. Growth, biofilm formation and cell viability of Xf strains from subsp. *fastidiosa* (7 strains), *multiplex* (18 strains), *pauca* (3 strains) and *sandyi* (2 strains) of different geographic and host origin have been determined in microplates with PD3 medium incubated at a constant temperature range of 6–32 °C at 4 °C intervals. Growth was determined daily by optical density at 600 nm, and biofilm was assessed at the end of the experiments by a crystal violet assay. Both parameters were modelled using a beta function to estimate the cardinal and optimal temperature values for each strain. Our results indicate that both growth and biofilm formation is determined by temperature and Xf strain. Overall, minimum values for both parameters were observed at the extreme temperatures of 6 and 32 °C. However, the range with optimal values largely depends on the Xf strain. Although no distinct range was associated with subspecies, the widest temperature range for optimal growth was estimated for Xf subsp. *multiplex* strains at 20–32 °C. Optimal growth was estimated at 24–32 °C for Xf subsp. *fastidiosa* strains. No clear pattern was found for Xf subsp. *pauca*, with optimal values between 16 and 24 °C or 20 and 28 °C, depending on the strains. Similarly, for Xf subsp. *sandyi*, optimal growth occurred at 16–28 °C or 20–28 °C. With a few exceptions, similar response to temperature was observed for biofilm formation. These results would allow the development of region-specific epidemiological models to predict the risk associated with the establishment and spread of Xf strains in Europe.

#### Acknowledgement

This work has received funding from 727987 XF-ACTORS (EU-H2020), E-RTA2017-00004-C06-02 from AEI-INIA Spain and FEDER, and the Spanish Olive Oil Interprofessional.

### Developing a spatial epidemiological model to estimate *Xylella fastidiosa* dispersal and spread

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**Abstract:** Understanding the dispersal of *Xylella fastidiosa* is essential for effective management of the disease. In Puglia, Italy, surveillance is focused on buffer and containment zones, which have been established at the edge of the infected region with the aim of containing further spread. Success of this strategy will strongly depend on whether these zones are wide enough to form a barrier to long-distance dispersal of the bacterium. In this presentation, I will describe our progress towards estimating the dispersal range of *Xylella* in Puglia using a generic spatial epidemiological model adapted

to the biology of the pathosystem. The model simulates the spread of the disease across a heterogeneous landscape depending on the location and timing of introduction, the distribution of host plants, the rate of infection growth in infected olive groves and both short- and long-distance dispersal. Long-distance dispersal seems to be a crucial feature of the *Xylella* epidemic, causing rapid spread of the disease over large areas but in an unpredictable manner. To try to estimate long-distance dispersal, we use Approximate Bayesian Computation to calibrate the epidemiological model to observed detections in surveillance monitoring data from 2013 to 2018. I will present results from the model calibration, comparing long-distance dispersal estimates from models specified for different long-range dispersal mechanisms. This will inform discussion on the roles of mechanisms such as vehicle transport and wind dispersal in spreading *Xylella* at regional scales.

### **An epidemiological model for the short-range spread of *Xylella fastidiosa* and the assessment of eradication management measures**

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**Abstract:** Early detection of the new outbreaks of *Xylella fastidiosa* and knowledge of the disease dynamics are key elements for an effective management of new foci. These elements were explored in the *Update of the Scientific Opinion on the risks to plant health posed by Xylella fastidiosa in the EU territory*, recently published by EFSA. In this opinion, a short-range spread model was used to investigate the spatial dynamics of a new outbreak in a free area and to comparatively assess the control measures aimed at local eradication of the disease. A process-based approach was used to describe the bacterial growth in a plant in relation to the symptoms/disease progression, the population dynamics of the spittlebug and the vector-mediated transmission. The model parameters were derived from the data acquired on the spread of *X. fastidiosa* subsp. *pauca* in olive groves in the Apulia region. Four epidemiological scenarios were considered combining host susceptibility and vector abundance. Four management options were considered to account for the timeline for the detection and for the implementation of control measures, efficacy of vector control and plant removal. Simulation results showed that the spread rate of the disease increases over time with a non-linear pattern depending on the scenario components. High efficacy of nymph and adult vector control and short delay in detection and implementation of control measures are key factors for the successful eradication of an outbreak in a free area. Model structure and flexibility make it possible to explore a wide range of conditions to account for different vector species, bacterial strains, vegetation components, landscape structures (homogeneous, heterogeneous but continuous, patchy) and combinations of management options. This makes the model a suitable tool to support decision making for the drafting and management of emergency plans related to new outbreaks.

### **Risk maps and risk-based surveillance for early detection of *Xylella fastidiosa* in continental France**

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**Abstract:** Recent detections of infected plant samples in the French regions of Corsica and Provence-Alps-Côte d'Azur rise the concern of a possible dissemination of the disease to other areas of continental France, where susceptible crops with high economic value are intensively cultivated, such as vineyards, olives and almonds. In this work, we develop a machine learning approach in order to predict the risk of infection for all continental France at a fine spatial scale. The proposed species distribution model learns from past observations of the disease and tries to understand which and how abiotic factors correlate with the presence of the bacteria. Provided with more than 100 possible explanatory

variables, our model consistently chooses a reduced set of environmental variables, such as water stress and mild winter temperatures, that are known from laboratory studies to enhance the probability of plant infection and the survival of the insect vector. The model is then used to predict the susceptibility of the entire territory, highlighting the potential risk of infection in previously unsuspected zones. Finally, we will also assess and measure the uncertainties associated with our predictions, based on the fact that the model is trained on a rather restricted geographical area (with mostly Mediterranean climate), that may not be representative of the entire national territory.

### **The spread of *X. fastidiosa* subsp. *pauca* among the olive orchards of southern Italy (Apulia)**

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**Abstract:** In 2013 an outbreak of *Xylella fastidiosa* was identified for the first time in Europe, in the extreme South of Italy (Apulia, Salento territory). The locally identified subspecies turned out to be lethal for olive trees, starting an unprecedented phytosanitary emergency for one of the most iconic crops of the Mediterranean area. The Apulian monitoring programme of the epidemic amassed data on several hundreds of thousands of samples laboratory-screened for the presence of the bacterium, jointly with georeferenced sample information. Starting from these data, it is possible to show that *Xylella fastidiosa* spreads by forming new, tightly clustered groups of infected plants (epidemic hotspots), with 98% of the infected trees separated by less than 100 m from another infected tree. Surprisingly, more than three quarters of the newly detected epidemic hotspots are farther than 1 km from any previously known infected plants. Considering this finding, either long-range spreading of the bacterium is underestimated, or the current monitoring strategy must be called into question. In both cases, however, it can be anticipated that, under the current monitoring protocol, yearly epidemic spreading 1 to 15 km far from olive trees currently labelled as infected will be more common than previously thought.

## Session 5: Vectors II

### **Insights into the transmission dynamics of *Xylella fastidiosa* by *Philaenus spumarius***

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnte 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** The establishment and relentless spread of the bacterium *Xylella fastidiosa* in some areas in Europe call for effective containment measures based on sustainable control strategies. However, the development of such strategies requires a thorough characterisation of the reciprocal interactions among the three key factors of the pathosystem, i.e. the bacterium, the vector, and the host plant. One of the major differences between European and American or Taiwanese epidemics refers to the vector species driving bacterium spread. Indeed, while sharpshooters (Hemiptera: Cicadellidae) are the key vectors in all the *X. fastidiosa* outbreaks other than European ones, spittlebugs such as *Philaenus spumarius* seem to play the main role in bacterial spread in Europe. Currently, knowledge about *X. fastidiosa*-spittlebug interactions and the characterisation of the mode of transmission considerably lags behind the background on sharpshooters. Here we began to fill this knowledge gap by carrying out EPG (Electrical Penetration Graph)-assisted transmission tests of *X. fastidiosa* by *P. spumarius* (acquisition from

infected olive plants and inoculation of healthy olives and oleanders). Furthermore, we conducted comparative observations on the probing and feeding behaviour of infective versus non-infective spittlebugs on healthy olive plants. Spittlebug acquisition rate of *X. fastidiosa* from olive appeared to be extremely low; bacterial cells binding to the foregut occurred in a time as short as 15 minutes spent by the insect in xylem ingestion or activities interspersed with xylem ingestion (interruption during xylem ingestion and resting). Either in olive or oleander, *P. spumarius* inoculation of bacterial cells into the xylem was associated with an early (2.5 to 7 minutes after the onset of the first probe) and occasional behaviour, visualised by a specific DC-EPG waveform (Xe), presumably related to egestion of fluids regulated by pre-cibarial valve fluttering following a lack of phagostimulation. Behaviours stereotypically repeated by the insect and commonly performed during most of the probes did not lead to bacterial inoculation to the host plant. Infective spittlebugs compared with non-infective spittlebugs exhibited: i) significantly longer non-probing and shorter xylem ingestion; ii) longer duration of single non-probing events; iii) fewer sustained ingestions (ingestion longer than 10 min) and interruptions of xylem activity (N); iv) longer time required to perform the first absolute probe. These observations suggest difficulties in feeding for infective *P. spumarius* probably caused by the presence of *X. fastidiosa* in the foregut. Overall, our findings open new perspectives for research on the *X. fastidiosa*–spittlebug relationship and for sustainable control strategies based on the disruption of bacterium–vector interaction.

### **A barcode database to identify the vectors of *Xylella fastidiosa* in Europe**

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**Abstract:** Fast and reliable identification is a critical point for the early detection of biological invasions. The research unit, CBGP (Centre for Biology and Management of Population), develops morphological and molecular tools to characterise pests and beneficial organisms associated with crops, with a strong focus on quarantine pests and invasive arthropods. After the first detection of the bacterium *Xylella fastidiosa* (Xf) in Corsica four years ago, we started to work on a reference barcode database to identify putative vectors of Xf. Our final goal in the framework of the H2020 project XF-Actors, was to decipher the interaction network between plants, vectors and Xf to get a better understanding of how the disease spread in the ecosystems. COI barcodes were generated through a two-step PCR approach followed by Miseq sequencing. Multiple quality controls were implemented including i) a non-destructive DNA extraction that allows vouchering and morphological re-examination by taxonomists; ii) a bioinformatic pipeline that discards non-coding sequences, contaminants and NUMTs through BLAST comparison and phylogenetic inferences.

While a reference library of validated barcodes is mandatory for a reliable identification of the vectors of *Xylella*, our first results reveal taxonomic issues: e.g. i) the closely related species *Philaenus spumarius*/*P. tessellatus* have identical COI sequences; ii) misidentifications are present in international databases.

All barcodes have been implemented in a web-interfaced database (Arthemis DB@se: <http://arthemisdb.supagro.inra.fr/DefaultInfo.aspx?Page=Home>). Currently, the database hosts 376 sequences of putative vectors of *Xylella*: 260 representing 21 European species and 116 representing 15 North American species. Users can identify a query sequence to species through BLAST comparison against our reference library. Metadata associated with all records are available online, as well as biological data on vectors and pictures that illustrate habitus, genitalia and diagnostic characteristics.

This data set is continuously supplemented and updated. Several species are still missing but it already enables the identification of the most frequent vectors of *Xylella*. A call is made to obtain the species not yet included in the database.

## Detection, identification and surveillance of *Xylella fastidiosa* on vectors in France

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** *Xylella fastidiosa* was identified in natural conditions in France in 2015, first in Corsica Island, then in Provence-Alpes-Côte d'Azur administrative region (French Riviera). Forty-nine host plants have been identified nowadays, infected by *X. fastidiosa* subsp *multiplex*.

In France, the detection protocol on vectors is based on the real-time PCR Harper et al. (2010) after a high-throughput DNA extraction based on the QuickPick™ Plant DNA kit used with a KingFisher™ robot. This method was evaluated in 2016–2019 mainly on *Philaenus spumarius*, the most effective vector based on POnTE studies from CNR. This protocol showed excellent performance criteria on individuals and groups of insects, on spiked macerates of insects from healthy areas and on naturally infected insects from outbreaks areas.

Strain identification was performed using MLST scheme (www.pubmlst.org) with the PCR Yuan et al. (2010) (modified EPPO, 2019) for amplifying sequences of 7 housekeeping genes. A protocol with addition of BSA into the PCR reaction mixture has been validated, showing improved performance in terms of sensitivity and success for strain typing. The centralisation of surveillance data at Anses enabled the production of zonal maps of the distribution of strains according to host species. They have been compared to compilation of results of detection and strain identification on insects *Philaenus spumarius* from Corsica and French Riviera regions. A good correlation between both maps has been observed in the same area. This work highlights the complementarity of the approaches on plants and insects in the framework of the epidemiological survey of the disease in a context of high risk for Europe.

## Transmission characteristics of *Xylella fastidiosa* subsp. *pauca* (ST53) by *Philaenus spumarius* and *Cicadella viridis*

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** For insect-borne plant pathogens, transmission biology is of major importance in outlining the disease epidemiology. The characteristics of acquisition, persistence and transmission of *X. fastidiosa* ST53 by the spittlebug *Philaenus spumarius*, the main vector in Apulia, are not yet described; similarly, transmission competence of the potential vector *Cicadella viridis*, the most common sharpshooter in Europe, is unknown. In this perspective, two sets of experiments were performed in 2017 and 2018 to study: i) the kinetics of bacterial multiplication and persistence in *P. spumarius* and *C. viridis*; ii) the influence of temperature, season and age of *P. spumarius* adults on simulated epidemic progression on olive plants under indoor and outdoor conditions.

For the kinetics experiments, following the acquisition, insects were serially transferred in groups of five to olive or periwinkle test plants. In simulated epidemic progression experiments, after the acquisition, groups of insects were isolated in cages with 16 olive seedlings for different inoculation periods. Acquisition and transmission rates were assessed by testing individual insects after inoculation and by testing recipient plants 6

and 10 months post-inoculation. Furthermore, acquisition and transmission of *X. fastidiosa* ST53 by *C. viridis* were tested through an *in vitro* acquisition system. Overall, about 900 insects and 170 plants were tested in kinetics experiments, while about 800 spittlebugs and 1,500 plants were studied in simulated epidemic progression experiments.

Preliminary results for *P. spumarius* indicate: a) a higher acquisition efficiency in September than July; b) a lower acquisition efficiency from periwinkle compared with olive as source plants, but higher transmission efficiency to periwinkle compared with olive as recipient plants. *Cicadella viridis* was able to acquire and transmit *X. fastidiosa* following acquisition on artificial diet or periwinkle, although with low efficiency.

### **Flight behaviour of *Philaenus spumarius*, the main vector of *Xylella fastidiosa***

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**Abstract:** *Xylella fastidiosa* jeopardises key crops in Europe. *Philaenus spumarius* was identified as the predominant vector involved in the spread of *X. fastidiosa* in southern Italy. This meadow spittlebug is also distributed in other regions, including Spain. Understanding vector dispersal ability is essential to predict the spread of *X. fastidiosa*. This insect species was reported to travel as much as 100 m within 24 hours in the field (1). However, both *P. spumarius* and *Neophilaenus lineatus*, another potential vector, can vertically displace up to the planetary boundary layer so they may passively travel long distances by laminar air currents (2). Our goal was to study the movement of *P. spumarius* under laboratory conditions using a modified commercial flight mill. Field studies on vector movement using vertical sticky traps; directional malaise traps and capture-mark-recapture techniques are underway. Different biotic and abiotic factors affecting the flight behaviour of *P. spumarius* are being studied: gender, adult age, temperature, light, barometric pressure, seasonality, geographic origin and rearing conditions among other factors. The individuals were tested using flight mills to estimate the number of flights, flight duration and number of turns (distance). The number and duration of turns were recorded using two different procedures: 1. Ethovision XT (Noldus), placing a video camera above the flight mill; 2. Mill\_recorder, a computer-based device programmed to register the number and duration of each turn. Our data available to date show that *P. spumarius* is able to fly a distance of at least 1.99 km in 1 h 40 min in a single flight, which is much higher than was previously thought. Furthermore, our preliminary results show that there are differences in the flight potential between males and females and between young and old adults. This knowledge on the flight potential of *P. spumarius* will be critical to improve management actions against the vector and the spread of *X. fastidiosa* in Europe.

### **Host plant affiliation of xylem-feeders in central Europe**

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnte 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** Provoked by the first notification of *Xylella fastidiosa* in Europe, several countries aimed to acquire data on the presence of already confirmed as well as potential vector species of the bacterium present in Europe.

In Germany, vector surveys were carried out predominantly on plant species which are economically important in central Europe and known to be highly endangered by *X.*



*fastidiosa* in the event of further bacterial spread, like grapevine, almond and cherry. Results are shown, which have been obtained from a three-year survey in the course of the project Xf-actors. The population dynamics of xylem-feeding species (spittlebugs, froghoppers and sharpshooters) were assessed in orchards (cherry and almond) and vineyards. Furthermore, the xylem-feeding activity of the most prevalent species on these plants was observed by EPG (Electrical Penetration Graph) to evaluate their potential vector-related risk as host for *X. fastidiosa* acquisition. The results will support an estimation of the transmission risk to specific crops under field conditions with a special focus on species able to transmit the bacterium, like *Philaenus spumarius* and *Neophilaenus campestris*.

## Session 6: Risk and impact assessment

### Monitoring the impact of *Xylella* on Apulia's olive orchards using Sentinel-2 satellite data and aerial photographs

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**Abstract:** Official surveillance of the *Xylella fastidiosa* (Xf) epidemic in Apulia shows how its front has moved northwards since 2013. However, assessing and modelling the impact of Xf remains difficult because little is known about the damage to the landscape behind the front lines.

Sentinel-2 and other satellite sensors provide images of the Earth in wavelengths that are sensitive to the vegetation condition. To test their suitability for Xf damage mapping, we created a model that relies on the shift that Xf-affected olive orchards undergo from a tree-dominated landscape to one dominated by shallow-rooted, short-statured vegetation. This transition changes the way gross primary productivity, as approximated by vegetation indices, responds to meteorological conditions. *In situ* estimates made by plant pathologists in olive orchards confirmed that a new method based on satellite and meteorological data detected Xf damage severity ( $R^2 = 0.6$ ). Furthermore, this method can be used to map Xf damage every year across Apulia.

We then tallied the individual olive trees lost since the first detection of Xf in 2013. We used aerial photographs from the summer of 2013 to delineate each olive tree in the region and photos of 2015 and 2018 to determine how many of them are now crownless, or gone altogether. We trained a Mask Region Convolutional Neural Network to delineate individual olive tree crowns in the 4-band photographs and it proved capable of accurately identifying crowns, irrespective of their size, shapes, proximity to neighbouring trees, or image resolution.

Our results provide a unique view on the spread of Xf in Apulia over the past five years, a means of systematically monitoring Xf damage across orchards, and an estimate of the number of olive trees lost thus far. It illustrates how remote sensing can provide a quantitative baseline for addressing the environmental and economic damage of Xf.

### An updated assessment of the risks to plant health posed *Xylella fastidiosa* in the EU territory

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**Abstract:** EFSA received a request from the European Commission to update its 2015 Pest Risk Assessment (PRA) for *Xylella fastidiosa* in the EU territory. The update focused on potential establishment, short and long-range spread, the length of the asymptomatic

period, the impact of *X. fastidiosa* and an update on risk reduction options, accounting for the different subspecies and Sequence Types of *X. fastidiosa* during the risk assessment where data availability allowed. Here we summarise the main conclusions of the updated PRA, the uncertainties associated with the conclusions, and recommended future directions for research and data collection to reduce these uncertainties. The quantitative PRA provided new insights on containing existing outbreaks and preventing further spread in the EU. For example, it was demonstrated that most of the EU has potential climatic suitability for *Xylella* but that the southern EU is most at risk; however, *X. fastidiosa* subsp. *multiplex* demonstrated areas of potential establishment further north in Europe compared with other subspecies. Modelling was also used to assess data from a wide range of studies on the length of the asymptomatic period, as well as to simulate the spread of the disease and management measures aimed at eradicating or containing the pathogen. The assessment showed the importance of disease control measures, including the use of buffer zones and plant removal, but also the need to couple these measures with effective vector control, rapid implementation of management measures following disease discovery, and early detection of new positive cases. The latter issue is a particular challenge for *Xylella* given the length of the asymptomatic period, particularly for some host and subspecies combinations. In a separate mandate EFSA is developing new surveillance guidelines for *Xylella* to enable for more effective and targeted detection surveys for the disease.

### **Potential impact of *Xylella fastidiosa* subsp. *pauca* in European olives: a bio-economic analysis**

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnte 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** *Xylella fastidiosa* is the causal agent of plant diseases which cause massive economic damage (Almeida, 2016; Chatterjee et al., 2008). In 2013, a strain of *X. fastidiosa* subsp. *pauca* was for the first time detected in Italian olives (European Food Safety Authority, 2015; Saponari et al., 2016). Here, we simulate future spread of the bacteria based on climatic suitability modelling and an assumption of radial range expansion. An economic model computes impacts by accounting for discounted foregone profits and losses in investment. The model computes impacts for Italy, Greece and Spain as these countries account for around 95 per cent of the European production (Eurostat, 2016). Climatic suitability modelling indicates that, depending on the suitability threshold, 92.5 to 95.4, 88.6 to 89.5 and 85.8 to 98.5 per cent of the national areas of production fall into suitable territory in Italy, Greece and Spain, respectively. Across the elicited rates of radial range expansion (Bragard et al., 2019), the potential economic impact over 50 years ranges from 3.58 to 8.69 billion euro if replanting with resistant varieties is not feasible. If replanting is feasible, the impact ranges from 2.00 to 4.13 billion euro. Depending on whether or not replanting is feasible, between 0.67 and 1.64 billion euro can be saved over the course of 50 years if the spread is reduced from 5.18 km to 1.1 km per year (50% and 5% percentile of elicited spread rate). The analysis highlights the major economic benefits of replanting with resistant olive cultivars and spread control. This stresses the necessity of strengthening the ongoing research on resistance traits and vector control.

### **Estimating the economic, social and environmental impacts of European priority pests: a joint project for EFSA and JRC and the case study of *Xylella fastidiosa***

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**Abstract:** Article 6 of the new EU Plant Health Law (Regulation 2016/2031) sets out the requirement to establish a list of priority pests from the list of EU quarantine pests. The prioritisation is built on the severity of economic, social and environmental impacts that those pests could cause in the EU territory. The European Commission asked the Commission's Joint Research Centre (JRC) to develop a multi-criteria decision analysis to rank pest species based on their potential multiple impacts, and asked EFSA to support the JRC's work by collecting the existing evidence and relevant data for 28 pest species regarding their potential capacity for establishment, economic and environmental impacts and difficulty of eradication. EFSA ran expert knowledge elicitations with groups of experts in order to obtain quantitative assessments and uncertainty analyses for four of the indicators: yield and quality losses, spread rate and time to detection.

Using data provided by EFSA, the JRC has developed a methodology to support the establishment of a priority pest list based on the soundest scientific evidence for those pests to which resources would be best allocated based on their impact severity. The Impact Indicator for Priority Pests (I2P2) is a composite indicator that ranks plant pests and pathogens according to their economic, social and environmental impact in the EU territory. The I2P2 should be applied to all quarantine plant pests and pathogens that can potentially affect EU agricultural and forestry sectors. This project represents a step forward in quantitative methodologies for pest risk assessment, respecting the need for more tiered and fit-for-purpose approaches in response to the needs of EU risk managers. *X. fastidiosa* was included in the list of species that were assessed and its results will be presented.

### **Living with *Xylella*: The dynamics of knowledges within *X. fastidiosa* 'sociopathosystems' in Puglia and Corsica**

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**Abstract:** Public management of bio-invasions, such as plant pathogens like *Xylella fastidiosa*, is not uniquely based on scientific knowledge, but instead on the conjunction of different necessities and the coexistence of different, sometimes competing, social actors and groups. The detection of *X. fastidiosa* in the EU territory has triggered mandatory measures that have sometimes generated strong reactions among different actors and groups such as researchers, stakeholders, local administrators and civic-environmental social movements. The understanding of such dynamics between different actors and groups in the management of bio-invasion is something that requires special attention. Some authors have pointed out the importance of incorporating approaches from the social sciences into phytopathological research, to interact with local knowledge, and to reflect on the social effects of phytosanitary policies. Starting from the analysis of two different case studies, namely, the diffusion of *X. fastidiosa multiplex* in Corsica (France) and the diffusion of *X. fastidiosa pauca* in Puglia (Italy), our contribution frames three different, but undeniably communicating, analytic 'layers' that would serve to perform a symmetric comparison between both cases and to highlight their differences and commonalities. Such layers will be: (i) legal/policy (the political management of *X. fastidiosa*, regulations, etc.); (ii) scientific knowledge production (the scientific study of the different strains of *X. fastidiosa* present in the EU and their interactions with different hosts and vectors); and (iii) mobilisations of stakeholders and civic-environmental social movements (mobilising for/against management policies, or mobilising for a reformation of scientific practices and knowledge production). Using qualitative methodological approaches and theoretical

concepts and from science and technology studies, our aim is to frame the social dynamics emerging from our cases as *X. fastidiosa* 'socio-pathosystems'. We will suggest that framing plant health management in this manner could result in better scientific, political, and socio-anthropological alternatives to image a harmonious coexistence with the bacterium *X. fastidiosa*.

## Session 7: Surveillance

### Targeting surveillance for *Xylella fastidiosa* in Europe: an epidemiological basis

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** The emergence of *Xylella fastidiosa* in Europe has highlighted the importance of surveillance for protection of host plants against exotic pathogens. However, the surveillance strategy will depend upon the surveillance aim – whether this is declaring pathogen absence, preventing pathogen establishment, containing and delimiting new pathogen incursions, or mitigating pathogen impact. Our previous work has also demonstrated that the biological characteristics of the pathosystem and the performance and costs of the methods used to detect the pathogen must be considered when planning and evaluating surveillance. We can achieve this by linking epidemiological models of pathogen spread (to predict where and when disease will occur) with statistically-informed sampling models, and by using mathematical or computational strategies to interrogate the output. We describe here how to implement surveillance in areas where *X. fastidiosa* is thought to be absent, including the 'uninfected zone' of Apulia, Italy, and the United Kingdom. Surveillance in these areas must be implemented for long durations of time, and therefore must be efficient and sustainable. It is well recognised that surveillance costs can be reduced while maintaining an acceptable probability of pathogen detection by precise targeting of surveillance efforts, but the question remains of how exactly to achieve this. For example, how should surveillance resources be balanced between sampling hosts and sampling vectors? Where should surveillance resources be placed in a large and heterogeneous landscape or trade network? And which detection methods should be used to detect the pathogen? We demonstrate how these questions can be answered using a variety of different spatial and non-spatial approaches. As well as providing specific recommendations for improving surveillance activities for *X. fastidiosa*, these methods are generic and thus can improve our understanding of surveillance systems in general for a range of pests and pathogens.

### Spatiotemporal monitoring of *Xylella fastidiosa* in olive trees using radiative transfer models and Sentinel-2 images

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**Abstract:** Detecting and monitoring the spatial and temporal dynamics of the symptoms and the severity of the damage caused by *Xylella fastidiosa* (Xf) is a key priority to prevent its expansion. This study evaluates the use of Sentinel-2 imagery together with a radiative transfer (RT) approach to monitor epidemics caused by Xf in olive trees. A time series of Sentinel-2a imagery collected over two years was used to describe the temporal dynamics of Xf-infected olive orchards located in the region of Apulia (southern Italy). Airborne hyperspectral acquisitions were used for validation along with field visual surveys carried out for more than three thousand trees with different disease incidence (DI) and severity

(DS) levels. A careful evaluation of the sensitivity of Sentinel-2 imagery to canopy alterations produced by a progressive Xf infection in olive orchards has been accomplished based on model simulations and field observations.

Our results demonstrate that the assessment of Xf infection monitoring based on Sentinel-2 data requires the use of self-corrected vegetation indices (VIs) and RT modelling. Among the tested VIs, those that minimise the atmospheric and background effects such as ARVI, ATSAVI and OSAVI performed better than traditional vegetation indices used as a quantitative proxy measure of the fractional cover (FC) of green and healthy vegetation such as NDVI, RDVI or MSR. Model simulations and field observations showed that the background effects have a significant impact on the temporal variation of DI levels detected with Sentinel-2a imagery. The use of 3-D RT modelling improved the DI estimates by 25% when accounting for the background effects, and by 32% when its heterogeneity was also considered. Therefore, the methodology proposed using a 3-D RT and Sentinel-2 data can provide useful spatiotemporal indicators to track the damage caused by Xf infections across large areas.

### **A survey in Israel reveals the presence of *Xylella fastidiosa* in almond trees in the northern part of the country**

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** *Xylella fastidiosa* is an insect-vectored, Gram-negative bacterial pathogen that until recent years was thought to be geographically restricted to the Americas. There, *X. fastidiosa* caused, and still causing, significant damage particularly to the grapevine and citrus productions in North and South America, respectively.

In 2013, *X. fastidiosa* was first reported outside the Americas, and was found in association with symptomatic olive trees in southern Italy. *X. fastidiosa* was later proven as the responsible agent for the devastating epidemic in southern Italy's olive groves leading to the olive quick decline syndrome. Later, *X. fastidiosa* was also reported from France mainland and Corsica, and from Spain mainland and Mallorca, causing disease in multiple host plants including *Polygala myrtifolia*, almond, cherry and grapevines.

In a survey conducted in Israel during 2017–2018, leaf scorching symptoms were observed in almond trees in the northern part of the country. Upon emergence from winter dormancy trees appear symptomless, although they tend to suffer from visible loss in foliage mass. From May onwards, the infected plants develop typical leaf scorching and curling symptoms. Both molecular and immunological assays showed that *X. fastidiosa* is associated with these symptoms. Multilocus sequencing analysis of several *Xylella* isolates, revealed that the dominant sequence type (ST) found in almond trees in northern Israel is ST1. Suspected olive and grapevine trees, showing scorching symptoms, were also tested and found negative for the presence of *Xylella*. Grafting experiments with *Xylella*-infected almond scions and artificial inoculations of healthy almonds, led to the appearance of typical disease symptoms. These results confirm the presence of *X. fastidiosa* in almond trees in northern Israel. Further studies to understand the breadth of the damage and to search for possible insect vectors are underway.

### **Optimization of the delimiting survey strategies for *Xylella fastidiosa* in the demarcated area in Alicante**

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** *Xylella fastidiosa* (Xf) is a regulated quarantine plant pathogen in the EU. The current legal provisions specify the implementation of delimiting surveys in infested areas to demarcate the geographic extent and implement eradication or containment measures. North-eastern Alicante Province, Spain, is one of the areas infested by Xf in the EU, and so it is currently demarcated and subject to a delimiting survey activity. Based on the 2018 official surveillance programme, in which approximately 100,000 ha were surveyed and 11,000 samples were taken and analysed, this work aimed: i) to estimate the spatial variation in disease prevalence; and ii) to improve the efficiency of the current delimiting survey plan.

Prevalence was modelled by means of a Bayesian spatial hierarchical model in which climatic and spatial factors were evaluated using INLA approximation. A delimiting strategy was designed based on a three-phase adaptive approach in which the surveyed area (epidemiological unit) size and sampling intensity were tailored according to the previous phase information. An algorithm was implemented to optimise the number of epidemiological units to be surveyed and the sample size by simulating different random sampling scenarios from the reference data. The strategy was evaluated by comparing the delimitation efficacy and prevalence estimates between the proposal and the reference data. Prevalence results revealed a strong effect of the spatial component in disease spatial variation. The proposed delimiting strategy achieved to delimit similar extension disease (similar efficacy) with a lower number of samples (better efficiency) than current.

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## EFSA tool kit for *Xylella fastidiosa* surveys in the EU Member States

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**Abstract:** The European Commission requested EFSA to support the EU Member States in the planning and execution of their survey activities for quarantine plant pests. In particular, EFSA is asked to provide scientific and technical guidelines in the context of (i) the new plant health regime (Regulation (EU) 2016/2031), in which prevention and risk-targeting are given an extra focus, and, (ii) the European Commission co-financing programme of the annual Member State survey activities for pests of EU relevance (Regulation (EU) No 652/2014). EFSA is currently preparing a toolkit for risk-based surveillance, including practical factsheets for ca. 50 pests and risk-based survey guidelines for three plant pests. *Xylella fastidiosa*, together with two other plant pests, is one of the organisms selected to develop and test these tools for EU risk-based surveillance. This presentation describes the principles of a risk-based approach, and the data requirements for performing a statistically sound sample size calculation, with *X. fastidiosa* as case study. Special focus is given to the *X. fastidiosa* risk-based guidelines, the design of the annual detection surveys and the related delimiting surveys implemented following a positive finding.

## Session 8: Biology and pathogenicity II

### Spatial distribution and Genetic structure of *X. fastidiosa* subsp. *pauca* in olive trees in south-east Brazil

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**Abstract:** The bacteria *Xylella fastidiosa* (Xf) is considered to be a non-specific plant pathogen, infecting a hundred plant species and causing disease in dozens of them. Different subspecies are known whereas the Xf subsp. *pauca*, Xf subsp. *multiplex*, and Xf subsp. *fastidiosa* are known to cause diseases in economically important crops in the Americas and recently in Europe. In South America, Brazil, the subsp. *pauca* is known mainly to cause the Citrus Variegated Chlorosis (CVC) in sweet orange, but was also described as causing the olive quick decline syndrome (OQDS) disease, specifically in the Southeast region, so far. The total area occupied by olive trees in Southeast Brazil is no more than 2000 ha which are located at high altitudes with small and dispersed orchards surrounded by native vegetation. We investigated, by PCR and isolation on BCYE medium, the geographic distribution of bacteria by analysing samples from 24 different orchards present in São Paulo and Minas Gerais States. Also, the population structure of 158 Xf isolates obtained from olive plants located in 10 different geographic regions was analysed by 12 Single Sequence Repeats (SSR) loci multiplexed in four sets for the PCR and electrophoresis. The forward primers were labelled with different dyes and the amplicons run by capillarity electrophoresis. Xf was detected in 20 out of 24 sampled orchards (83%). Of a total 158 isolates, 64 different multilocus microsatellite genotypes (MLMGs) were observed, i.e. 40%. Unbiased Nei's genetic diversity corrected by the population size (H<sub>Nei</sub>) index ranged from 0.00 to 0.55 (AVG of 0.24 for all populations), lower than obtained by subsp. *pauca* populations from citrus and coffee (Francisco et al., 2017). F<sub>ST</sub> index (Wright's fixation index) for all pairwise comparison of populations ranged from 0.075 (p>0.01) to 0.968 (p<0.001), meaning a strong subdivision among some populations, even with the recent outbreak. Discriminant Analysis of Principal Components (DAPC) and PCoA via Nei Unbiased Genetic Distance clustered the samples into three mainly genetic groups.

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### Cultivar susceptibility and temperature-dependent recovery of *Xylella fastidiosa*-infected grapevines

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**Abstract:** Previous research in California demonstrated that *Xylella fastidiosa* (Xf) can be eliminated from infected grapevines and almond trees by exposure to cold winter temperatures. Duration and temperature of winter conditions, degree of host plant susceptibility, and time between inoculation and cold exposure are believed to play a role in the rate of pathogen persistence and disease reoccurrence. Initial studies of over-winter survival of Xf in grapevine primarily focused on climate conditions experienced in northern California, and included a limited range of cultivars. To better understand the dynamics of vine recovery from Xf infection following cold stress, grapevines of three different cultivars were subjected to cold treatment at 4°C after either 8 or 16 weeks post-inoculation. Plants

that underwent cold treatment at 8 weeks post-inoculation were separated into two groups; one inoculated in June, and one inoculated in late August to evaluate the effect of accelerated symptom development due to hot mid-summer temperatures. All vines were tested with qPCR prior to cold treatment to determine infection status. Following 8 weeks of cold treatment, all vines were grown back from dormancy for 20 weeks and evaluated for reoccurrence of disease by symptom observation and qPCR testing. In this study, cultivar susceptibility and higher temperatures during initial infection stages had a greater impact on disease reoccurrence than duration of infection prior to cold treatment. This suggests that use of tolerant or resistant plant material should be a priority for areas at risk of Xf infection, and that summer temperatures need to be considered in addition to winter temperature and time of inoculation, when gauging likelihood of vine recovery. Further information regarding the effect of climate factors such as temperature on pathogen persistence is important to inform region-specific management strategies, and to evaluate the risk of Xf spread in new areas.

### **Phenotypic characterisation of two Spanish strains of *Xylella fastidiosa* subsp. *multiplex* ST6 differing in plasmid content**

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**Abstract:** Two *Xylella fastidiosa* (Xf) subsp. *multiplex* strains IVIA5901 and ESVL were isolated from symptomatic almond trees in Alicante (Spain). Even if these Xf strains show an average nucleotide identity at the chromosomal level of 99.99%, they differ by the presence of two plasmids pXF64-Hb\_ESVL and pUCLA-ESVL, only found in strain ESVL. Xf colonisation and disease development in host plants have been shown to be related to the size of cell aggregates, bacterial motility and biofilm formation, which are mediated by type I and type IV pili, among other traits. The goal of this study was to characterise the phenotypic characteristics of these strains that may be related to their differences in plasmid content.

The Spanish Xf subsp. *multiplex* strains were compared with Xf subsp. *multiplex* strains Alma-Em3 and BB08-1 isolated from blueberry and the reference strain Xf subsp. *fastidiosa* Temecula1 from grapes, all isolated in the US. To study bacterial behaviour and phenotypic characteristics, several experiments were performed to determine adhesion force to substrate, biofilm formation, movement, cell-cell aggregation, twitching motility and patterns of bacterial growth. Additionally, a virulence assays were conducted in the greenhouse using tobacco plants cvs. SR1 and Xanthi. We also determined the presence of genes coding for type I and type IV pili in the Spanish strains. Our results show that the two Spanish isolates of Xf subsp. *multiplex* have lower motility, less capacity of aggregation, make less biofilm, and cause lower disease severity when compared with the US isolates used in this study. Also, there were significant differences in disease severity between Xf Spanish strains that varied according to the tobacco cultivar, with Xanthi being the most susceptible one. Besides, our results indicate that strain ESVL exhibits stronger attachment to substrate than IVIA5901, but no differences in biofilm formation, cell-cell aggregation or twitching motility were found between them. The role of plasmids in the biology of these Spanish Xf strains will be further investigated.

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### **Ancestral state reconstructions of *Xylella fastidiosa*–host plant relationships**

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**Abstract:** The broad host specificity that *X. fastidiosa* exhibits globally contrasts with the increased plant host specificity for individual strains. Understanding the molecular underpinnings of plant host specificity in *X. fastidiosa* is vital for predicting host shifts and epidemics. While there are multiple genetic determinants of host range in *X. fastidiosa*, there should still be detectable genomic evidence of the unique relationships between *X. fastidiosa* and its hosts. The objective of this project is to use phylogenetics to predict the ancestral plant hosts of *X. fastidiosa*. We used genomic data to construct phylogenetic trees of subsets of the core and accessory genomes at varied clade depths. With those trees, we created maximum likelihood (ML) ancestral state reconstructions of plant host at several taxonomic scales (species, genus, and multi-order clade). While some genomic regions were not historically informative in terms of predicting ancestral host state, others predicted high likelihoods of particular ancestral plant hosts at ancestral nodes. In future work, these same historically predictive genome regions could be used to identify genetic underpinnings of host specificity and be integrated into modelling potential host jumps and host range changes of individual strains of *X. fastidiosa*.

### **Rethinking the *Xylella fastidiosa* scenario in the Balearic Islands: what epidemiological, phylogenetic and dendrochronological data tell us**

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**Abstract:** The emergence of *Xylella fastidiosa* (Xf) in Europe has been dealt as relatively recent introductions from the American continent. While this seems accurate for highly virulent genotypes such as the strain currently causing the quick olive decline syndrome in Apulia since ca. 2013, less virulent Xf genotypes might have gone undetected for a long time, being confused with drought or fungal disease symptoms under Mediterranean climatic conditions. Indeed, the current widespread incidence and severity of the Pierce's disease (PD) and Almond Leaf Scorch Disease (ALSD) in Mallorca Island can only be understood in this context of a 20-year introduction scenario. Our current epidemiological, phylogenetic and dendrochronological data on Xf strains belonging to subsp. *fastidiosa* ST1, and Xf subsp. *multiplex* ST81, causing Pierce's disease (PD) and ALS, respectively, strongly suggest that they were overlooked for decades. Both subspecies were very likely transported from California to Mallorca with infected almond scions around 1995 and subsequently spread throughout the island by the local vector *Philaenus spumarius*. Our phylogenetic analysis based on WGS of isolates of both subspecies from Mallorca supports their Californian origin. Congruent with this, Xf DNA was consistently detected in the growing rings of infected almond trees from 2006 to the present and occasionally as far back as 1998. In the main focus in Son Carrió more than 50% of the almond trees died and 90% of trees showed symptoms compatible with ALS in 2012, but the aetiology of this problem was attributed to fungal trunk diseases, drought or field abandonment. We hypothesise that during the last 20 years, Xf subsp. *multiplex* ST81 isolates have adapted to wild olive trees, widespread on the island, causing a mild dieback, and later on reaching the island of Menorca very likely on infected *P. spumarius* transported as a hitchhiker on ships moving between the islands.

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## Session 9: Sustainable control measures

### The VSPP, a voluntary certification programme to produce healthier plants for planting in the EU

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**Abstract:** The Voluntary System Preventing Pests (VSPP) was developed within Task 9.4 of the XF-Actors project by a group composed of experts from national scientific organisations, industry, NPPOs and international organisations. It aims at better integrating plant health risks in the production of plants for planting. This voluntary certification system is firstly based on the implementation in companies of quality management processes. Certified companies must also apply general plant health requirements for the prevention of pests, including the identification of critical points in the production chain. In addition to these general requirements, technical requirements for the prevention of specific pests (initially *X. fastidiosa*) can be applied for selected plant species and to a specific category of plants. These pest-specific requirements could later be extended to other pests that may represent a risk for the industry in the EU. This voluntary system builds upon the current EU and national regulations and proposes additional requirements for EU *X. fastidiosa*-demarcated areas (e.g. analysis of critical points) as well as for the rest of the territory (e.g. initial testing for all host plants and production under insect-proof facilities for the whole production chain). General rules of the VSPP have been developed taking into account the minimum requirements for a pest risk management plan as described in Article 91 of Regulation (EU) 2016/2031. Therefore, any participant certified according to the VSPP certification programme will be regularly audited, which could facilitate the approval of its pest risk management plan by the National Plant Protection Organizations (NPPO). The participant may then be subject to plant passport inspections with a reduced frequency. A first draft Standard is finalized and is now shared more widely with other stakeholders. The possible organisation of the system is also being discussed.

### N-acetyl-cysteine for controlling *Xylella fastidiosa* in citrus and olive: understanding the differences to improve management

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**Abstract:** A new approach to control Citrus Variegated Chlorosis (CVC) caused by *Xylella fastidiosa* is the use of N-acetyl-cysteine (NAC). CVC symptoms include reduction in fruit size making it not useful for juice or fresh fruit consumption. To analyse the effect of NAC under field conditions, severe CVC symptomatic plants were treated with NAC during two season harvests (2013–2014 and 2014–2015). A significant increase in fruit diameter was observed in the two seasons in plants treated with NAC-fertiliser, improving its quality for commercialisation. A new field trial was carried out during seasons 2017–2018 and 2018–2019 with similar results to those observed before. Based on the results of NAC on CVC, some field trials were performed in southern Italy to verify its effect on olive quick decline syndrome (OQDS), a novel olive disease caused by a highly pathogenic strain of *X. fastidiosa* subsp. *pauca*. The assessment was made based on the level of symptoms on branches (dieback and dessication) of olive trees selected in plots of a highly susceptible cultivar. In general, treatment with NAC seems to decrease the disease progression,

especially using NAC-endotherapy. These results were more evident in the trials set up on the plots with low initial incidence of symptomatic trees, where for three years (from 2016 to 2018) some differences were recorded. But, in 2019 as the pressure of inoculum increases in the environment, an increase in disease progression was observed in all treatments. Taken together, as also observed for CVC, NAC does not completely control the bacterium, but does interfere with the progression of the disease. However, in CVC-treated plants, NAC leads to an improvement in fitness of diseased plants during the time of treatment, which was not observed for olive. Some considerations must be pointed out when comparing CVC and OQDS pathosystems. The disease in olive (and in particular in the highly susceptible cultivar used in our experiments) is more severe than in sweet orange, for which no plant death is observed. This suggests that citrus may have a more efficient plant defence response that, along with the effect of NAC, may improve disease resistance. Similarly, it is worth extending the NAC applications to infected olives of less susceptible/resistant cultivars, in the attempt to strengthen the innate host response with the positive effect of the NAC application. In addition, in commercial citrus orchards the control of vectors helps to prevent new infections and consequently the use of NAC may be more effective.

### **Further acquisition on the response of a large number of olive cultivars to infections caused by *Xylella fastidiosa* subsp. *pauca*, ST53**

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**Abstract:** Deployment of resistant varieties is a key strategy for mitigating economic losses due to *Xylella fastidiosa* infections in crops, particularly in those areas where the bacterium finds favourable conditions for its persistence and infections are associated with severe diseases. As such, genetic traits that confer resistance/tolerance have been identified in the past in grapes, citrus and, more recently, in olives. Although understanding the molecular pathways involved in the resistance is a primary target of many studies, nowadays supported by the powerful resolution of the high-throughput sequencing technologies, biological confirmation remains a paramount tool to ultimately define the response of a given plant genotype. In this context, the emergence of *X. fastidiosa* in olives in southern Italy, prompted for large surveys and biological screening tests of olive germplasm in open fields and in greenhouses. Test plants are monitored for symptoms, incidence of bacterial infections, estimation of the bacterial population size and, more recently, analysed for the expression of LRR-RLKs- and ABA-related genes. The results so far collected highlighted the following aspects: (i) under controlled conditions the latent period of the infections in olives is as short as one year in the most susceptible cultivars, whereas under field conditions a first reliable estimation of the symptoms associated with the infections could be retrieved upon at least three years of exposure to the natural pressure of inoculum; (ii) cultivars under testing could be firstly distinguished in highly susceptible' those that developed symptoms in the shortest time and those 'potentially tolerant/resistant' deserving further investigation; (iii) upon needle-inoculations or field exposure, cultivars could be differentiated based on the rate of systemic infections, as highly infected vs low-infected. Interestingly, several cultivars either in greenhouses or in field testing produced results similar to those recorded for the cultivar Leccino, used as resistant control; indeed cultivars harbouring high bacterial titers but not showing symptoms (i.e. with traits of tolerance) have also been identified. Although preliminary, these studies allowed to gather information on the spectrum of tolerance/resistance of additional olive cultivars.

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## **Strategies for reducing vector populations and transmission of *Xylella fastidiosa* in olive groves**

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**Abstract:** Much of the work regarding insecticidal efficacy against *Philaenus spumarius* has been initiated only in the past few years, when the control of this spittlebug species in the area where outbreaks of *Xylella fastidiosa* emerged, became essential. Among the numerous formulations (synthetic and organic insecticides) tested to control the adults on olives, a major affected European crop, neonicotinoids and pyrethroids showed the highest efficacy and persistence (Dongiovanni et al., 2018). These trials were extended in 2018 and 2019 by testing a new formulate based on acetamiprid and one based on cyantraniliprole, a systemic insecticide belonging to anthranilic diamide. For both, results showed high efficacy against *P. spumarius* indicating they could be adopted for controlling spittlebug populations in the framework of the application of containment measures for *X. fastidiosa*.

Whereas, with the purpose of reducing the efficiency of *X. fastidiosa* vector transmission under organic farming management, applications of kaolin were tested for four consecutive years as a preventive approach to protect a new olive plantation exposed to the natural inoculum pressure. Although, applied on a calendar basis, the use of kaolin did not protect the young olives from infections and subsequent symptoms development. Surprisingly, this was also the case of the plants treated with the insecticide used as control, based on imidachloprid, for which, even if to a lower extent, infections also occurred.

Attempts were also made to implement strategies, alternative to the mechanical control of weeds, for controlling the juveniles, a stage of the insect life when they are more vulnerable and control can be more efficient. Sowing different gramineous species to replace the natural ground vegetation, applications of herbicides and pyroherbicides, were compared. Soil tillage, pyroherbicides and herbicides applied in spring were the only interventions able to reduce almost to zero the presence of juvenile spittlebugs.

The experimental data herein developed will be helpful for the end-users to choose better options for the management of this vector in different agroecosystems.

## **Understanding the olive microbiome of susceptible and resistant cultivars for sustainable biocontrol**

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**Abstract:** Olive Quick Decline Disease (OQDS) is a devastating olive disease, which emerged few years ago in the region of Apulia (southern Italy) as a result of the bacterial infections caused by *Xylella fastidiosa* subsp. *pauca*. Bacterial infections were consistently associated with severe desiccations on the local cvs Cellina di Nardò and Ogliarola salentina, whereas mild symptoms were found in the infected trees of the cvs Leccino and

FS17, indicating that these cvs may harbour traits of resistance. Investigations on the olive microbiome of OQDS-resistant and susceptible cultivars were undertaken to identify potential protecting endophytes for a sustainable strategy of biocontrol. In this study, bacterial and fungal communities of the xylem of infected trees of the cvs FS17 and Kalamata, respectively symptomless and highly symptomatic, were analysed by barcode (16 S rRNA V4 and ITS1-spanning amplicons) and whole shotgun (WSS) sequencing. Overall, the core microbiome was dominated by fungi, accounting 99.8% and 88.4% of the total reads by barcode and WSS sequencing, respectively, while Proteobacteria and Ascomycota are the most represented phyla with both techniques. This fungi/bacteria ratio was maintained in all trees of the cv FS17 while it was inverted in the susceptible cv Kalamata, in which *Xylella* colonised the majority of the ecological niche in the heavily infected plants. Bacteria were isolated from the sapwood of olive trees of the cv Kalamata and FS17 and plate assays were performed to evaluate the antagonistic activity of these olive endophytes against *X. fastidiosa*. Moreover, bacterial communities of selected trees of the cv FS17 were isolated, purified and co-inoculated with *X. fastidiosa* into potted olive plants of the cv Cellina di Nardò for evaluating potential effects on the evolution and progression of *Xylella* infections in this susceptible cultivar. Data on antagonistic activity of isolated endophytes and the progress of *Xylella* infections and/or symptom appearance in these microbiome-Xf co-inoculated plants will be presented.

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### **Assessment of *Paraburkholderia phytofirmans* PsJN biocontrol potential against *Xylella fastidiosa* 'De Donno' strain in olive**

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**Abstract:** Numerous attempts have been made to test the use of endophytic bacteria to control diseases caused by *Xylella fastidiosa* (Xf), but promising results obtained *in vitro* have often proved inadequate once transferred to the field.

A new encouraging possibility came from the observation that the bacterium *Paraburkholderia phytofirmans* PsJN, widely studied for its ability to defend plants from biotic and abiotic stresses, was able to reduce the symptoms caused by Xf in grapes affected by Pierce's Disease.

Our study aimed at testing the effectiveness of PsJN as a biocontrol agent in the Xf strain 'De Donno'/olive pathosystem. Although *in vitro* tests showed the absence of competitive inhibitory effects of PsJN on Xf 'De Donno' growth or its ability to form biofilm, several trials were started both in greenhouse and in Xf-contaminated orchards. Despite different approaches were tested to deliver PsJN in olives, high rates of successful isolations and positive detection, achieved with a SYBR<sup>®</sup>-Green-based qPCR assay ad hoc developed in this study, were obtained only upon needle inoculation of 1- to 2-year-old shoots, in which PsJN proved to remain viable for a period of time >500 days.

Current observations in open-field trials, so far limited to a single season, have not revealed significant differences in the reduction of OQDS symptoms or Xf concentration in therapeutic treatments, between plants treated or not with PsJN, nor reduction of the new infections upon preventive applications. Despite the evidence that PsJN can colonise the

xylem vessels, time course diagnostic tests clearly showed that it moves slowly away from the point of inoculation and its concentration decreases significantly over time.

This absence of systemic colonisation suggests a possible induced response of the plant, which will need to be analysed in detail, to optimise treatments and trigger a cascading effect on Xf. In this direction, preliminary results of PsJN impact, on the resident microbiome diversity indices, in presence/absence of Xf, have been gathered using a WGS approach.

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# Poster sessions

## Session: Biology and pathogenicity

### Identifying *Xylella fastidiosa* host adaptation candidate genes: the case of *X. fastidiosa* subsp. *pauca* isolates and olive trees in Italy

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**Abstract:** The introduction of a *Xylella fastidiosa* (Xf) subsp. *pauca* strain in Italy has resulted in the first disease epidemic of this pathogen in Europe. We cultured the bacterium and performed whole-genome sequencing of over 70 Xf. subsp. *pauca* isolated from olive trees from 2013 to 2017 across affected areas in southern Italy. We identified several genes under positive selective pressure within the Italian population; these are genes that might be involved in the adaptation of *X. fastidiosa* to olive trees. Other aspects of epidemiological relevance, such as estimating the date of introduction of *X. fastidiosa* to Italy, are also being extracted from this dataset and will be presented.

### Current investigations on the susceptibility of potential host plants to *Xylella fastidiosa*, to evaluate the risk of introduction, establishment and spread in Belgium

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**Abstract:** *Xylella fastidiosa* was reported in 2018 in a Belgian nursery in West Flanders. Even before this event, the need to scientifically explore the threat to the country presented by the bacterium had been recognised, and investigation started in 2016. Based on a first screening of the *Xylella* host plants, three model tree species were selected: *Prunus domestica* cv Opal, *Quercus petraea*, and *Salix alba*.

The susceptibility to the bacterium of these plant species is investigated in two different ways. The first way, known as 'sentinel plantation', is the establishment of these plant species in areas contaminated by the bacteria to evaluate their susceptibility under natural conditions. Based on the presence of the largest number of *Xylella* subspecies and STs in the EU, the area selected was the campus of the University of the Balearic Islands (UIB). In total, 27 plants of each targeted species were brought from Belgium and planted in the UIB campus in a repeated randomised fashion. Additionally, 32 *Rosmarinus officinalis* plants were used as known susceptible host plant controls. A floristic inventory was carried out to survey host plants in the vicinity. Field surveys have been carried out regularly on the plantation and in the surrounding monitored area. Molecular methods including several PCR (Minsavage et al., 1994; Harper et al., 2010; Cruaud et al., 2018) were used for the bacterial detection in collected insects and investigated plants. The second complementary way consists in mechanically inoculating different strains of *X. fastidiosa* into the plant xylem in biosafety quarantine-controlled glasshouses. The presence and the progression of the bacterium into the plants is monitored by PCR as well as by confocal microscopy with the use of a GFP-marked strain kindly provided by S. Lindow (Newman et al., 2003). Both approaches of investigating susceptibility will be discussed.

## Functional comparative analysis of *Xylella fastidiosa* accessory and pan-genomes

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**Abstract:** Genome comparative analysis suggests that accessory elements are important drivers of evolution and differentiation in *Xylella fastidiosa* and related plant pathogens, thus providing useful information on bacteria virulence and environmental adaptation traits. In this work, we use the software AccNET (Accessory Genome Constellation Network) to qualitatively and quantitatively compare currently available *X. fastidiosa* genomes. This application allows phylogenetic and functional information to be combined by using the proteomes extracted from the analysed genomes, thus improving the capability to explore bacterial species pan-genomes.

The comprehensive whole-genome study provided by AccNET agrees with the previously published *X. fastidiosa* phylogeny, showing that it is effective in delineating the genetic relatedness of *Xylella* isolates. Comparison of the accessory genome established by using an arbitrary threshold of 30% with a hierarchical clustering dendrogram representing the similarity of accessory genomes was also congruent. Furthermore, our analysis showed that approximately half of the protein clusters were exclusive of 50% of the genomes, limiting the previously expected number of accessory elements in *Xylella* with the increasing number of available genomes. Thus, application of AccNET to *X. fastidiosa* genomes provides an important resource to inspect accessory homologous proteins and to identify new genetic determinants and subspecies biomarkers.

## Screening for resistance of grapevine cultivars to *Xylella fastidiosa* in Europe: a dead-end approach

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**Abstract:** The first established outbreak of Pierce's disease in Europe was detected on grapevines in Mallorca in 2017. Subsequent surveys showed that the disease was widespread and affected grapevine cultivars with variable incidence and severity depending on crop management and distance from the main focus (eastern Mallorca). Up to autumn of 2018, twenty-three different grapevine cultivars were positive to *Xylella fastidiosa* (Xf). To assess the response of grapevine varieties to Xf infection, we conducted a two-year inoculation experiment with isolate XYL 2055/17 (subsp. *fastidiosa*, ST1) on different scion-rootstock combinations in insect-proof tunnels under Mallorcan climatic conditions. Overall, 35 cultivars were included in the assays comprising common cultivars in the EU such as Merlot, Syrah and Chardonnay; Spanish popular regional varieties (e.g. Tempranillo, Garnacha, Verdejo) and local ones (e.g. Callet, Gorgollasa). We monitored biweekly the development of Pierce's disease from the 8th to the 16th week post-inoculation counting symptomatic leaves above and below the point of inoculation. All plants either symptomatic or asymptomatic, including controls, were tested for the presence of Xf by qPCR before and 12 weeks after inoculation. Our first-year results showed that almost all cultivar-rootstock combinations are susceptible to some degree to Xf. However, symptom development differed among cultivars over time and disease expression was modulated by the rootstock. On average, Xf moved 2.3 times faster towards the apex than towards the roots within the cane. Our data suggest that searching for cultivar resistance within common grapevine varieties is an unsuitable approach to fight against the Pierce's disease challenge in Europe. Rather, these types of assays should be focused on obtaining quantitative data in relation to the bacteria movement and multiplication within the plant over time and its effect on the acquisition and transmission by the insect vector.



## Host plant range of different *Xylella fastidiosa* subspecies in experimental tests

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**Abstract:** *Xylella fastidiosa* (Xf) is an insect-transmitted bacterium, which infects the xylem of a wide range of plants. This plant pathogenic bacterium is genetically diverse with five proposed subspecies that have different regions of origin and were once thought to have different host ranges. Since the first detection of Xf in Europe in 2013, various strains of at least four subspecies were reported. The De Donno strain from the subsp. *pauca* infects mainly olive trees, but has been recovered from more than 20 other plant species. Strains from the subspecies *multiplex*, *sandyi*, *fastidiosa* and *pauca* have been identified in Spain, in France and in the north of Italy infecting more than 60 plant species altogether. Large overlaps in host range of the various subspecies were noticed. However, these host lists came from analysis of naturally occurring infections that depends largely on uncontrolled factors such as host availability where the strain is present and the presence of insect vectors attracted by these plants. The potential host range of the different strains circulating in Europe or dread strains remain poorly understood. We tested the pathogenicity of seven strains belonging to different subspecies (*fastidiosa*, *pauca*, *multiplex* and *sandyi*) on a large range of plants of interest for French horticulture such as citrus, grapevine, olive trees, plum and apricot trees. Survival at the inoculation point, colonisation at a distant point, and development of symptoms were quantified after pin-prick inoculation and incubation in confined growth chambers over 6 to 18 months. Generally, in these conditions symptoms were difficult to quantify and analyse, except on grapevine. Strain ability to disseminate in the plant from the inoculation point varied for a same plant species upon cultivars. Survival at the inoculation point was also a differential characteristic among strain-plant combinations. Our results will be useful for risk management in Europe.

## Genomic analysis and biology of a novel variant of *Xylella fastidiosa* subspecies *multiplex* infecting different host plants in Tuscany, Italy

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**Abstract:** An increasing number of *Xylella fastidiosa* outbreaks associated with strains of the subspecies *multiplex* has been reported in southern Europe, currently the most common strains being detected in Corsica, in the Balearic Islands, in the province of Alicante (Spain) and, more recently, reported for the first time in Portugal and in the region of Tuscany (northern Italy). The Tuscany outbreak raised major concerns, being associated with a new variant of *X. fastidiosa* subsp. *multiplex*, characterised as sequence type ST87, capable of infecting, among the others, almond. Although this sequence type is closely related to the more common ST6 and ST7 variants, remarkable differences were recorded when culturing the ST87 isolates on different media as well as when comparing their draft genomes. In contrast to the majority of the strains of the subspecies *multiplex*, the ST87 isolates grow on PD3 solid medium, a peculiar feature described for a few and highly virulent strains associated with almond leaf scorch disease in North America (Almeida and Purcell, 2003). Analyses based on core genome alignments and single nucleotide polymorphisms of currently available *Xylella* genomes show they are genetically related to

strains previously characterised in North America, but in a separate clade from strains of the subspecies *multiplex* previously sequenced in Europe, supporting the hypothesis that they originate from a distinct introduction that occurred in Europe. Pathogenicity tests on *Prunus* spp. and grapes are ongoing to assess the virulence and the host range of this newly discovered variant of *X. fastidiosa*.

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### Identification of multilocus SSR markers to assess genetic diversity of *Xylella fastidiosa* subsp. *pauca*, ST53, spreading in Apulia (southern Italy)

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**Abstract:** The epidemic spread of *Xylella fastidiosa* in Apulia (southern Italy) with the continuous expansions of the border of the infected area, incited several investigations into the genetics and genomics of the strains associated with the infections. These studies were aimed to infer genetic correlations with known strains of the bacterium as well as among the initially discovered Apulian outbreaks and those that emerged from time to time. MLST and full genome sequencing provided clear evidence that a single genotype, denoted ST53, of the subspecies *pauca* was causing the epidemics on olives and other hosts. In this work, we tested a panel of SSR markers in the attempt to disclose information on the genetic diversity and evolution of the bacterial population, even if, given the reduced spatial and temporal scales of this recent epidemic, a relatively low level of variation was expected. Five SSR markers selected among those previously reported in the literature proved to be polymorphic for the ST53-isolates from Apulia, that even if yielding a small number of alleles (from 2 to 5), when combined provide a good resolution, distinguishing several genotypes. Additionally, the design of a new set of 12 SSR markers for fine-scale genotyping of the Apulian isolates, yielded higher number of allelic variation, paving the way to perform micro-evolutionary and epidemiological studies. Indeed, the successful use of these markers on DNA plant samples, will allow a large-scale study, taking advantage of a six-year dataset of plant DNA collected and stored in the framework of the official monitoring programme started in the region at the beginning of the epidemics in 2013 and covering the entire demarcated area in Apulia that currently exceeds 200,000 ha.

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### *Xylella fastidiosa*: what are the factors that make this bacterium pathogenic and host-specific?

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**Abstract:** *Xylella fastidiosa* is a gram-negative pathogen that causes disease in a wide variety of agriculturally significant plants, such as olive, almond and cherry trees, to name a few. The pathogen poses a great threat to the UK as many of its host plants are found here. The present study attempts to understand the genetics behind the different subspecies of *X. fastidiosa* by means of population genomics. There are currently 46 publicly available genomes. A pipeline is being developed to identify factors that aid in the virulence of the bacterium, which will then be implemented for a larger population.

This research may gain more insight into the complex host range of the bacterium, its yet unknown mode of action within the plant, and determine why the pathogen causes disease in some hosts but remains asymptomatic in others. Ultimately, the outcome of the study could pave the way to implementing further control measures and creating diagnostic tools for the prevention of an outbreak.

### **Screening olive germplasm for resistance to olive quick decline syndrome caused by *Xylella fastidiosa* under field and controlled conditions**

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**Abstract:** Searching for resistance is regarded as one of the most promising long-term control strategies against the olive quick decline syndrome (OQDS) caused by *Xylella fastidiosa* (Xf). A set of 60 olive genotypes are now being screened for resistance to OQDS under field and controlled conditions. Plants consisted of self-rooted plants, produced at IFAPA in Córdoba (Spain) and then transferred to the CNR-IPSP-Bari (Italy). A first batch of 10 genotypes from different *Olea europaea* subspecies and 10 breeding selections from the IFAPA breeding programme was planted in open field under high disease pressure in November 2016 and inoculated under controlled greenhouse conditions in March 2017. A second batch of 40 cultivars selected from the World Olive Germplasm Collection, representative of the genetic and geographical variability of the collection, was inoculated under controlled greenhouse conditions and exposed to natural infections in summer 2017. Preliminary results indicate differences in the incidences of the infections and, among those that support systemic infections, differences were recorded for the presence and severity of shoot dieback and desiccation phenomena. Results gathered under controlled conditions allowed to identify some genotypes in which poor systemic infections (only few replicates were colonised) were detected even upon two rounds of inoculations. Conversely, symptoms of shoot dieback were recorded on some accessions, most probably indicating they are particularly sensitive to the infections. Interestingly, the selection of Leccino confirmed previous evidence, with inoculated plants harbouring low bacterial population size and not showing severe shoot dieback. However, especially for the field experiments affected by the weather and climatic conditions, observations and quantitative assays need to be prolonged in order to acquire conclusive data from multi-year surveys. On the basis of these preliminary results, a first set of progenies from tentative resistant genitors are being currently developed for future studies.

### **Genomic insights into the diversity of European *Xylella fastidiosa* isolates**

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**Abstract:** A growing number of recent and current studies on *Xylella fastidiosa* outbreaks in Europe emphasise genomic sequencing of bacterial isolates as a mean to infer their

potential origin, biology, recombination events and possible determinants of host specificity.

In the present work we have undertaken a re-assessment of publicly available *X. fastidiosa* genomic sequences from a comparative standpoint. With limitations due to the fact that most of the available sequences were left at the draft stage, and that only a few are complete, we undertook to compare: i) genomic organisation (synteny); ii) number, origin and genomic position of insertion elements (IS, islands, phage-related elements, plasmids); iii) individual gene complements within the species and subspecies pan-genomes; and iv) exclusive or enriched genes associated with subspecies or host of isolation. Our preliminary results suggest that *X. fastidiosa* genomes show a great plasticity in their organisation, possibly aided by the widespread presence of insertion elements, and that the traditional subspecies classification, although useful from an operational standpoint, is probably insufficient to explain the observed genomic variability.

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## **A global map of *Xylella* plasmidome reveals broad host and geographical distribution**

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**Abstract:** Despite the knowledge generated on *Xylella fastidiosa* (Xf), the contribution of mobile genetic elements to its biology remains unveiled. Conjugative elements, in addition to its role in genetic transfer, are involved in bacterial adhesion and biofilm formation. One of the main difficulties to treat Xf is linked to its ability to form biofilms, both in the plant xylem and in the insect vectors. Previous studies reported the existence of different plasmids in Xf; however, their functions, distribution and host range have not been globally characterised. Here, by analysing plasmid genomic composition and sequence homology, we infer a global map of the *Xylella* plasmidome.

Our genomic analysis found that almost all Xf sequenced strains carry at least a potentially conjugative plasmid encoding a relaxase (belonging to the MOB<sub>P</sub> family), a coupling protein and a complete T4SS (generally belonging to the MPFT type). Furthermore, half of these plasmids harbour at least a virulence-coding gene, most of them putatively involved in cell adhesion. Analysis of Xf plasmids by average nucleotide identity levels unveiled that they organised into a few discrete coherent genomic clusters, that we called plasmid taxonomic units (pTUs). Interestingly, some clusters were restricted to a single Xf host, while other pTUs have colonised all subspecies, indicating that they can be directly exchanged and spread among them. The homologous protein network analysis showed the proteome specificities and commonalities of Xf pTUs. Considering that conjugation involves co-occurrence of donor and receptor cells in the same ecological niche, our analysis sheds light on *Xylella* evolutionary history and geographic distribution.

## **Phylogenomic analysis of the species *Xylella fastidiosa***

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**Abstract:** *Xylella fastidiosa* is a phytopathogenic bacterium considered as a quarantine pathogen for the EU that affects a broad spectrum of hosts. It has stood out in recent years due to its detection in the EU, especially in Italy, the Balearic Islands, Alicante and Corsica. It has important environmental implications and is the cause of millionaire agricultural losses.

It is a Gram-negative bacterium of the class Gammaproteobacteria, within the family Xanthomonadaceae. The genus *Xylella* nowadays is formed by two species, *X. taiwanensis*, recently described as a pathogen in pear trees, and *X. fastidiosa*, described in 1892 as a vine pathogen causing Pierce's disease. This last one is divided into six subspecies: *fastidiosa*, *multiplex*, *pauca*, *morus*, *sandyi* and *tashke*. Only subsp. *fastidiosa* and subsp. *multiplex* are subspecies validly recognised in taxonomy.

In order to infer the phylogenetic distribution of the different subspecies, all genomes available in the databases, together with different genomes of strains isolated in the Balearic Islands and sequenced in our research group were analysed. Sequence types were determined using the pubMLST website for *X. fastidiosa* (<https://pubmlst.org/xfastidiosa/>). Comparative genomic analysis was carried out using the GET\_Homologues software. *In silico* whole-genome comparison tools (ANIb, TETRA and GGDC) were also used. The results obtained by the different approaches clearly demonstrate a separation into three phylogenomic branches that correspond to the subspecies *fastidiosa*, *multiplex* and *pauca*.

### **Lack of evidence for seed transmission of *Xylella fastidiosa* subsp. *pauca* from infected olive trees and annual host plants**

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** In 2013, *Xylella fastidiosa* emerged in southern Italy threatening mainly olive trees, which upon bacterial infections succumb to severe desiccation and rapid decline. High rates of infected and symptomatic trees are usually recorded in the contaminated olive groves. Such evidence prompted several investigations to assess the pathways of local spread of the infections. Beside graft-/insect vector-mediated transmission, the possibility that the pathogen may be vertically transmitted through infested seeds was also investigated, by testing seeds collected from naturally infected olives and weeds (*Erigeron* spp. and *Chenopodium album*).

Four lots of olive fruits were harvested in January 2014 and 2016 from infected olive trees selected in three different locations in the Apulia region (southern Italy). Seeds were cleaned from the pulp and used either for the diagnostic tests (qPCR assays) or stratified at 4°C for three months followed by germination. For diagnostic tests, 24 seeds for each source were used to test either the excised embryos or the endosperm plus the seed coats.

Upon germination, the number of seedlings recovered varied between 30 and 50 for each lot, with a total of 160 seedlings grown in confined conditions for five years. Diagnostic tests on seedlings were performed one year after the germination and then repeated three (seeds collected in 2016) or five years (seeds collected in 2014) later.

Similarly, for the infected weeds diagnostic tests were performed (i) on groups of seeds (>100 seeds/sample) harvested in 2016 from infected plants, and (ii) on six-month-old plantlets obtained after seed germination.

The results of the qPCR assays on the seeds and on the recovered seedlings (both for olives and weeds) unequivocally indicated lack of positive detections, supporting the evidence of lack of seed-to-seedling transmission of this bacterium as previously shown for other susceptible crops (Della Coletta-Filho et al., 2014).

### **Studies to elucidate the cause of alteration in colony morphotype of *Xylella fastidiosa* subsp. *pauca*, ST53**

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**Abstract:** Isolation in pure culture of *Xylella fastidiosa* (Xf) strains from infected olives showing severe decline in southern Italy, represented a major breakthrough for advancing the research on this first and most severe bacterial outbreak which emerged a few years ago in Europe. However, during an extensive isolation campaign, some of the cultured isolates (Xf subspecies *pauca*, ST53) started to show an odd shape and growth pattern. Briefly, after an initial growth, the colonies started to become translucent, of reduced size, roughness and adherent to the media. Passage of these isolates on new agar-plates, regardless of the media, evolved in cell death. Microscope examination using the BacLight LIVE/DEAD bacterial viability staining kit and observations at the Transmission Electron Microscopy (TEM) revealed that the odd-shaped bacterial colonies consisted of dead cells, and the presence of different bacteriophage-(like) particles were observed in the TEM preparations. Ultrathin sections of these bacterial colonies showed a higher number of intracellular vesicles/endospores than those observed in the cells with regular morphotype, and their content discharged into the extracellular space. Filtrated suspensions prepared from altered colonies scraped from the plates, were able to reproduce similar alterations when mixed with ST53-isolates (subsp. *pauca*) of the regular morphotype. Conversely, no effects were noticed when strains belonging to other subspecies were put in contact with the same filtrates.

Studies are in progress to understand the nature of these intracellular vesicles/endospores which resemble a sort of 'pseudolysogenic phages' (Ripp S and Miller RV, 1997) induced in other bacterial species as a strategy to enhance survival when unfavorable environmental conditions occur. Indeed, attempts to purify phage particles from cultures displaying odd colonies are ongoing to verify whether they originate by spontaneous induction from temperate phages, known to occur in the genomes of the ST53-isolates causing the infections in the epidemic area of Apulia (southern Italy).

#### Acknowledgement

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#### Bibliography

Ripp S and Miller RV, 1997

## The updated and interactive European Food Safety Authority database of *Xylella* spp. host plant species

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**Abstract:** In 2013, following the first outbreak of *Xylella fastidiosa* in the European territory, the European Commission requested advice to the European Food Safety Authority (EFSA). EFSA conducted a pest risk assessment and created a database of host plant species of *X. fastidiosa*. Since then, the database has been further improved and the last update, published in September 2018, included information on host plants of both *X. fastidiosa* and *X. taiwanensis*. A systematic literature review was performed and data were extracted from more than 850 publications allowing the compilation of a list of 312 host plant species detected by two highly reliable detection techniques, with a total of 563 host plant species regardless of the detection method. Additional information on botanical classification, infection conditions, geographic location, pathogen taxonomy (including information on subspecies, strain and sequence type), and tolerant/resistant response of the host plant was also extracted. This systematic approach will be repeated regularly to keep the host plant database up to date. The raw data were published on the 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 researchers, risk assessment and risk management and main results will be presented.

### **Evaluation of vascular occlusions in xylem vessels of olive cultivars infected with *Xylella fastidiosa***

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**Abstract:** Understanding the mechanisms underlying the development of symptoms of Olive Quick Decline Syndrome (OQDS) and the different olive cultivar responses to infections caused by *Xylella fastidiosa* subsp. *pauca*, ST53, is fundamental for disease control. The combined action of bacterial aggregates and plant-derived vascular occlusions (tyloses, gums and gels) has been invoked as the cause of the pathogenic alterations occurring in *Xylella*-infected plants. Conflicting observations were found about the role of vascular occlusion in the disease progression in field-grown olives. In the present work the distribution of vascular occlusions in the secondary xylem vessels of susceptible, Cellina di Nardò, and resistant, Leccino and FS17, cultivars were studied by light microscope observations of toluidine blue stained sections, recovered from stems of greenhouse-grown mock and artificially inoculated olives. One-year-old shoot portions collected from symptomatic or symptomless twigs were firstly tested by qPCR and used to recover thin sections (0.2 mm thick), with a similar number of vessels inspected for each cultivar. In the sections recovered from the non-infected controls 0.15%, 0.02% and 0.13% of occluded vessels were present, respectively in Cellina, Leccino and FS17. These percentages increased in the infected twigs reaching 9.65%, 6.81% and 1.33% in Cellina, Leccino and FS17, respectively, indicating that *Xylella* infections (regardless of the cultivar) induced occlusions of the xylem vessels; these were significantly higher in the susceptible cultivar. More specifically, percentages of occluded vessels in the susceptible cultivar ranged from 1% to 34%, while those of the resistant Leccino and FS17 ranged from 0.044% to 14% and 1.09% to 1.53%, respectively. Although a clear-cut difference was observed among the infected cultivars, within each cultivar no significant differences were recorded between symptomatic and non-symptomatic shoots, suggesting that the development of symptoms is enhanced by a combination of different factors (anatomical, chemical and physical).

### **Antibiotic susceptibility and virulence profiling of endemic *X. fastidiosa* subsp. *fastidiosa* isolates from Costa Rica**

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**Abstract:** *Xylella fastidiosa* subsp. *fastidiosa* is endemic in Costa Rica. Although the bacterium has great potential for disease and it is widespread throughout the country, a common feature is that infected host plants tend to be asymptomatic or in the worst case scenarios show mild symptoms as reported for 'crespera disease' in coffee. Another trait within this population is that isolates have shown broad genetic diversity, based on different typing techniques including MLST. Our recent efforts have focused on phenotypic characterisation of isolated strains. We tested antibiotic susceptibility of *X. fastidiosa*

subsp. *fastidiosa* isolates to tetracycline, streptomycin, gentamicin, chloramphenicol, ciprofloxacin and penicillin. Minimal Inhibition Concentration (MIC) ( $\mu\text{g/ml}$ ) were generated using Etest® assay, a quantitative technique that has been used previously in slow-growing bacteria including *X. fastidiosa*, with reproducible results; a difficult task to achieve using other susceptibility determination techniques. Results show an overall susceptibility of the isolates to most of the antibiotic tested; however, differences in MICs within isolates for each antibiotic should not be overlooked. To evaluate whether the endemic *X. fastidiosa* subsp. *fastidiosa* population is capable of infecting and causing severe disease in *Nerium oleander* as seen for *X. fastidiosa* subsp. *pauca* (ST53), also present in Costa Rica, several isolates of *X. fastidiosa* subsp. *fastidiosa* representing different ST types, were assayed in a virulence test using *Nerium oleander*. Eight months after inoculation none of the tested isolates have induced evident symptoms of disease and bacteria were barely detected near the inoculation site six months post-inoculation. Monitoring is still ongoing.

This research was financially supported by funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635646: POnTE (Pest Organisms Threatening Europe) and grant agreement No 727987: XF-ACTORS (*Xylella fastidiosa* active containment through a multidisciplinary oriented research strategy).

### **Leaf ionome profile of susceptible and resistant olive cultivars infected by *Xylella fastidiosa***

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** *Xylella fastidiosa* (Xf) is continuing to emerge as a devastating bacterial pathogen for many economically relevant species. Xf subsp. *pauca* strain De Donno is associated with Olive Quick Decline Syndrome (OQDS), a destructive disease occurring in the southern area of Apulia (Italy). In susceptible olive cultivars, symptoms are characterised by initial leaf scorch and scattered desiccation of small branches that over time worsen and extend to the whole canopy. Greenhouse and field observations revealed that olive trees of the cultivar Leccino show milder symptoms, when compared to those observed in Ogliarola salentina. Ogliarola salentina have progressive, severe scorching and complete dieback. Moreover, the lower bacterial population size in Leccino confirmed the resistance of this cultivar to Xf infection, suggesting that it is able to limit pathogen multiplication.

To understand the role that mineral nutrition may play in host resistance to OQDS, a field survey of the leaf ionome was carried out in trees of two orchards located in the Xf-infected demarcated area, that showed clear differences in response to Xf infection. Infected leaf samples, classified as symptomatic and asymptomatic, were subject to the determination of the ion content by inductively coupled plasma – optical emission spectrometry (ICP-OES). Data were analysed in relation to the different cultivars and the presence or absence of symptoms.

The comparison between symptomatic and asymptomatic samples showed an increase of sodium levels in both cultivars and significantly higher calcium levels in the symptomatic tissues of Leccino, a response that had been found in other Xf-host pathosystems. Otherwise, Leccino trees had a significantly higher content of manganese, in both symptomatic and asymptomatic leaf tissues.

These field observations inform currently ongoing experiments under controlled conditions to investigate the relevance of these mineral ion changes in the development and progression of symptoms, and the potential involvement of manganese in resistance of the Leccino cultivar to Xf infection.

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## Monitoring of biofilm production in *Xylella fastidiosa* strain De Donno via biochemical signalling modulation

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**Abstract:** Diffusible lipid species are exploited by bacteria for regulating cell motility, cell-to-cell communication, activation of metabolism and proliferation. The most widely investigated lipid family is represented by diffusible signal factors (DSFs) responsible for quorum sensing. *Xylella fastidiosa* (Xf) uses the DSFs to coordinate genes involved in the expression of virulence and biofilm formation. These moieties are mainly cis-2-unsaturated fatty acids which directly enhance xylem infection and biofilm production, which are the two processes involved in the genesis of severe plant diseases such as the noticeable Olive Quick Decline Syndrome, associated with Xf subsp. *pauca* strain De Donno and affecting olives in the Apulia Region. Here we report the results of studies aiming to identify DSFs molecules of Xf De Donno and exploit strategies for modulating its biofilm formation.

We started inducing DSF expression in *Escherichia coli* using a plasmid vector recombinant for the *rpff* gene of Xf De Donno and verifying the production of exogenous proteins and fatty acids. We set up extraction, mechanical treatments, and methyl ester derivatisation of the extracted crude oils from Xf and *E. coli* cultures. We compared the GC-MS profiles of fatty acids belonging to the metabolic activity of bacteria harbouring the bare and *rpff*-recombinant plasmids. Completing previous studies, we speculated on the production of unsaturated fatty acids with a chain length of 12-18 carbon atoms, with  $\alpha$ -unsaturated functions. Isolated and treated crude extracted oils obtained from the same bacterial sources, were tested *in vitro* to investigate their phenotypic effect on biofilm growth and the expression of key genes related to surface adhesion, biofilm formation and cell movement.

Furthermore, we set the synthesis of new, no commercially available, cis-2-unsaturated fatty acids with a chemical structure related to the DSFs family, in order to test the *in vitro* alteration of biofilm production in Xf De Donno. The exploited reaction was the stereoselective Still-Gennari olefination which leads to the synthesis of unsaturated fatty acids in cis (Z) conformation starting from commercial aldehydes.

Results of these activities will be presented.

## Experimental confirmation that *Xylella fastidiosa* subsp. *pauca*, ST53, does not colonise grapes

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**Abstract:** *Xylella fastidiosa* is able to colonise a very large number of plant species, but considering each subspecies/phylogenetic clade the number of associated susceptible hosts is significantly reduced. Although strains genetically related most likely share similar host range, using phylogenetic relationships to infer information regarding the potential host

range of new strains is still problematic, and pathogenicity tests remain the only means to assess the capability of a given strain to infect or not a specific plant species. Based on the current European legislative provisions on *X. fastidiosa*, information on the host range of the strain(s) causing an outbreak have regulatory consequences. In this context, we have made efforts to prove experimentally the capability of *X. fastidiosa* subsp. *pauca*, ST53, one of the most virulent European genotypes, to infect grapes, using 23 grape varieties (*Vitis vinifera*) and four rootstocks. Upon needle inoculation, plants were monitored for 18 months, using standard diagnostic methods (qPCR and isolation), supported by vector-transmission tests and observation of thin sections of the inoculated stems, stained using the LIVE/DEAD BaLight kit. qPCR assays on samples collected at the inoculation points (i.p.) and from the distal portions, 6 and 12 months post-inoculation, yielded positive reactions in more than 90% of the i.p., whereas in half of the cultivars scattered amplifications (the majority yielding Cq values > 30) occurred in some replicates at 15–20 cm from the i.p., but none of the apical portions tested positive. Isolations made 18 months after the inoculation, either from mature leaf petioles and stems portions harbouring the inoculation points, failed to recover actively growing colonies. At the same time, microscope observation of the thin sections showed only the presence of aggregates of dead *Xylella*-cells at the i.p. Transmission tests performed using specimens of *Philaenus spumarius* caged on the inoculated grapes produced negative results for both insects and recipient plants. The overall results showed that the bacterium was successfully delivered into the stem of the grapes and bacterial residual could be qPCR-detected even one year after the inoculation, but none of the inoculated cultivars sustained active bacterial multiplication and colonisation.

#### Acknowledgement

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### Transformation of *Xylella fastidiosa* subspecies *pauca* strain De Donno

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**Abstract:** *Xylella fastidiosa* subsp. *pauca* strain De Donno has been recently identified as the causal agent of a severe disease affecting olive trees in a wide area of the Apulia Region (Italy). While insights on the genetics and epidemiology of this virulent strain have been gained, the complex network of interactions with the main susceptible host remains to be explored. A fundamental tool for understanding such interactions is the development of bacterial mutants for functional analysis of genes involved in the host recognition, pathogenicity and insect transmission. Experimental studies have demonstrated the natural competence of *X. fastidiosa* in the uptake of exogenous genetic material; a feature exploited for site-specific introduction or deletion of genes through homologous recombination. Nevertheless, numerous studies have shown that several factors may affect *X. fastidiosa* transformation efficiency, including growth rate, twitching motility, sequence similarity, and the presence of Restriction–Modification systems that cleave incoming DNA. On this basis, two different plasmids containing the chromosomal replication origin (oriC) of *X. fastidiosa* and *E. coli* were used to transform *X. fastidiosa* De Donno in order to produce a GFP-expressing and a knockout strain for the *rpfF* gene, a crotonase producing a diffusible signal factor (DSF), involved in the quorum-sensing system. Repeated attempts to exploit natural competence, introducing the donor plasmids into *X. fastidiosa* De Donno failed, highlighting the critical role of genetic diversity in recombination performances of this pathogen. Conversely, GFP and RpfF mutants were successfully obtained by co-electroporation in the presence of an inhibitor of the Type I R-M system, that had been proved to impact the stable acquisition of foreign DNA by *X. fastidiosa* subsp. *fastidiosa*. Availability of mutants for one of the most virulent strains of *X. fastidiosa* opens for new explorations of host–microbe interactions, important to elucidate mechanisms

underpinning the differential responses recorded upon infections of different olive cultivars and toward the implementation of strategies to mitigate the impact of the disease.

#### *Acknowledgement*

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### **Occurrence of plasmids pXF64-Hb\_ESVL and pUCLA-ESVL associated with infections caused by *Xylella fastidiosa* subsp. *multiplex* ST6 in the demarcated area of Alicante, Spain**

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**Abstract:** An outbreak of *Xylella fastidiosa* (Xf) subsp. *multiplex* ST6 was first identified in June 2017 in Alicante province (Valencian Community, Spain) affecting mainly almond trees, but also other species including ornamentals, cultivated *Prunus* spp. and landscape plants. The current demarcated area (DA) in Alicante covers 134,581 hectares, and affects more than 70 municipalities. Genetic analysis may provide evidence of biological, ecological and host-range diversity among Xf strains of the same subspecies and STs and help to elucidate the pathway of introduction and spread in an affected area. Pairwise comparisons of the chromosomal genomes of two ST6-sequenced strains isolated from almond trees in Alicante, ESVL and IVIA5901, showed an average nucleotide identity higher than 99.9%. Interestingly, the two strains differ for the presence of the plasmids pXF64-Hb\_ESVL and pUCLA-ESVL detected only in the ESVL strain. The aim of this study was to determine the incidence and distribution of plasmid-bearing strains of Xf subsp. *multiplex* ST6 in Alicante. PCR tests were performed on 20 strains isolated from different hosts, and on approximately 100 DNA samples from infected almond trees collected in eight municipalities within the DA of Alicante. PCR results on the cultured isolates showed the occurrence of ST6-strains harbouring the two plasmids, or only the plasmid pUCLA-ESVL or none of them. Interestingly, in some of the DNA samples from infected almonds, only the plasmid pXF64-Hb\_ESVL could be detected. More specifically, 4% of ST6-infected samples harboured the plasmid pXF64-Hb\_ESVL, 12% only pUCLA-ESVL, 16% both plasmids, and 44% neither of the two plasmids. In five of the eight municipalities, we found samples harbouring the two plasmids, and only in the samples from the municipality of Benifato the PCR tests failed to amplify the targeted plasmids. Future work will increase the number of samples to cover the entire DA to better understand and relate the presence of plasmids to the epidemiology of this disease in combination with the use of multilocus sequence typing and genome sequencing of more Xf subsp. *multiplex* ST6 strains.

### **Olive tree dieback diseases in Tunisia and threat of quick decline syndrome caused by *Xylella fastidiosa***

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**Abstract:** Like in many Mediterranean countries, the olive tree is of great importance in Tunisia. However, due to the changes in the olive cultivation practices and horticultural techniques, a syndrome of dieback, wilt and death of young olive plantations has frequently occurred in the main olive-growing regions of Tunisia. The disease is characterised by rapid dieback of shoots, twigs and branches followed by death of the entire tree. In fact, previous studies of the disease aetiology based on pathogenicity tests, revealed the involvement of

some branch and soil-borne pathogens, such as *Cylindrocarpn destructans*, *Phytophthora* sp., *Pythium irregulare*, *Athelia rolfsii*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Armillaria mellea*, *Fusarium solani*, *Verticillium dahliae*, *Neonectria radicola*, *Neofusicoccum australe* and *Phoma fungicola*, in the occurrence of olive dieback and death in many olive groves. However, it was recently shown that the decline and death of the olive tree is not only caused by phytopathogenic fungi but also by bacteria such as *Xylella fastidiosa*. Although the disease is limited to olive groves in Italy and Spain nowadays, it represents a real threat for all the Mediterranean countries, where olive is extensively grown. In Tunisia, *X. fastidiosa* is regularly surveyed by inspecting visual symptoms and laboratory analyses of suspected samples by PCR and serological tests. Fortunately, the pathogen is not detected and typical symptoms of the disease were not revealed during routine inspections carried out in different olive-growing regions. In Tunisia, the Ministry of Agriculture has reacted to the occurrence of this disease by introducing new legislation to classify *X. fastidiosa* as quarantine pathogen. Other recent legislation restricts the introduction of host species from countries experiencing *X. fastidiosa* infection. In addition, a national committee was established to design an action plan including several measures to be applied in the absence or presence of the disease.

### **Olive crops from Argentina affected by *Xylella fastidiosa***

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**Abstract:** Decline symptoms in olive trees caused by *Xylella fastidiosa* (Xf) infection have emerged as a potential disease threat to one of the most important olive production areas in Argentina. In La Rioja province, the local cultivar Arauco is the most affected by the bacterium but it has also been detected in the cvs. Manzanilla, Arbequina and Picual. Since 2013, a marked decline of the traditional cultivar has been observed affecting both old and young orchards. A total of 80 samples were collected from infected trees in seven commercial lots of cv. Arauco. Some of them were randomly selected around the tree and others, sectorised from branches with a marked dried symptom advance. Xf was best detected from the sectorised samples (92%) than from the samples taken around the tree (65.8%) and so far, only the *pauca* subspecies (ST69) has been detected in olive plants. This type sequence has only been found in Argentina where it was also detected in orange trees from the north-eastern region. To identify the potential vectors of Xf, sampling was conducted in olive orchards positive to the bacterium located in La Rioja province (Chilecito). Since 2016, insects have been collected from olive trees with sticky yellow traps and from the surrounding vegetation with entomological sweep nets. The collected specimens were identified by Auchenorrhyncha specialists (CEPAVE, CONICET-UNLP and IMYZA INTA Castelar). Until now, the subfamily Cicadellinae (Cicadellidae) was the second most represented and species of the genera *Macugonalia* Young, *Scopogonalia* Young and *Molomea* China were the most abundant. The sharpshooters *Bucephalogonia xanthophis* (Berg), a vector of Xf in Brazil (Redak et al., 2004) along with other species that tested positive for the bacteria in north-eastern Argentina were also recorded. Specimens of *Notozulia entreriana* (Berg) (Cercopidae) were collected in the spontaneous vegetation associated with the crop.

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### **Characterisation of olive xylem microbiome community composition by metabarcoding greatly depends on the matrix used to extract DNA and 16S universal bacterial PCR primers**

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** Understanding of xylem sap microbiome is becoming of relevant importance for plant health as it could include microbes that may protect against xylem-limited pathogens, such as *Xylella fastidiosa*, and supporting key biological processes. Furthermore, the negative pressure, low oxygen and nutrient content of the xylem sap make it a unique and unexplored microbial environment. In this study, we evaluated the differences obtained in the characterisation of the xylem microbiome composition when using xylem sap extracted from xylem vessels using a Scholander pressure chamber or when using macerated fine chips obtained from xylem tissues from 10-year-old or 1-year-old olive trees. We also compared four different PCR primer pairs targeting 16S rRNA for their efficacy to avoid co-amplification of mitochondria and chloroplast 16S rRNA, as this suppose an important drawback in metabarcoding studies. PCR primers tested included 799F/1062 (V5-V6), 799F/1115 (V5-V6), 967/1391 (V6-V8) and 799F/1193 (V5-V7). Illumina paired-end sequence quality control and chimeric filtering was performed with DADA2 using QIIME2. Taxonomy affiliation into OTUs at 99% was based on the Silva reference database. The highest mitochondria and chloroplast amplification was obtained when using xylem chips and 799F/1062 (77.7%) and 967/1391 (99.6%) primers. On the contrary, 799F/1115 and 799F/1193 primers showed the lowest mitochondria (< 6.76%) and chloroplasts (< 0.02%) amplification, and the highest number of OTUs identified, 245 and 247, respectively. Interestingly, only 81/236 and 27/240 OTUs or 66/144 and 21/149 genera were shared between xylem sap or wood shavings after amplification with 799F/1115 or 799F/1193, respectively. The most abundant bacterial genera (> 50% of reads) included *Anoxybacillus*, *Cutibacterium*, *Methylobacterium*, *Pseudomonas*, *Rathayibacter*, *Sphingomonas* and *Spirosoma*. However, their relative importance varied depending of the matrix and primer pairs used. These results will help to optimise analysis of xylem microbiome community composition and more importantly to understand its driving and modifying factors.

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## Session: Detection and surveillance

### Estimating the asymptomatic period of *Xylella fastidiosa* from incomplete data

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**Abstract:** Visual detection remains the primary frontline method of detection of *Xylella fastidiosa* infection in susceptible host plants. Surveillance systems for detecting and delimiting new incursions and for declaring pathogen absence from areas considered free of the pathogen are therefore impacted by the duration of the 'asymptomatic period' between infection and the first emergence of disease symptoms. However, relatively few data are available on the asymptomatic period of *X. fastidiosa*, especially considering the wide variety of bacterial subspecies and strains in different host plants and climatic conditions. Following a request from the European Commission as part of the recent EFSA

update of the 2015 *Xylella* Pest Risk Assessment, we developed a novel strategy for estimating the duration of the asymptomatic period from data routinely recorded during experimental infection studies. Using the timing and numbers of symptomatic and asymptomatic hosts at first symptom development and the end of the study, we are able to estimate the probability of a given host not having developed symptoms at any given timepoint following infection. We do this using two methods: one assuming that symptom development occurs at a fixed rate over time (allowing us to extrapolate beyond the available data); and another which makes no such assumption (and thus more accurately represents the data available). Using data obtained from a comprehensive review of the literature on experimental inoculation studies, we found good agreement between both methods. Although care should be taken when interpreting the results, we are able to draw useful conclusions regarding the duration of the asymptomatic period for different *X. fastidiosa* subspecies in different hosts. Importantly, we find that almond infected with the *multiplex* subspecies, and sweet orange or olive infected with the *pauca* subspecies, remained asymptomatic for the longest durations (up to five years) after infection, with implications for ongoing surveillance activities.

### **Experience of *X. fastidiosa* diagnostics in symptomatic and asymptomatic plant material in the Russian Federation**

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**Abstract:** *Xylella fastidiosa* infects a huge number of plants, among which there are species of agricultural importance to the Russian Federation. The Laboratory of Bacteriology of FGBU 'VNIKR' conducted testing of domestic commercial kits of reagents for the extraction of *X. fastidiosa* DNA from the tissues of infected symptomatic samples. In the course of the research the following kits were tested: 1) Proba-GS (OOO AgroDiagnostika); 2) Proba-NK (OOO AgroDiagnostika); 3) DNK Ekstran-2 (ZAO Sintol); 4) K-SORB (ZAO Sintol). Based on the results of the studies, a significant efficiency of the Proba-NK reagent kit for DNA extraction from leaf and petiole tissues was established. In order to extract DNA of the pathogen from lignified parts of plant samples, the SORB-GMO-B reagent kit (ZAO Sintol) was tested, which contains CTAB ionic detergent as a lysing agent and provides maximum DNA yield from plant components.

Later on, the pathogen DNA obtained from infected samples was used to determine the sensitivity of various tests based on PCR and real-time PCR on 10 DNA dilutions. Based on the results of these studies, a modified real-time PCR in accordance with Harper et al. (2010; erratum 2013) and the 'Fitoskrin *Xylella fastidiosa* – RT' commercial kit are recommended.

Well-tested methods of DNA extraction and PCR tests were used for scientific monitoring in the Republic of Crimea and the city of Simferopol. Seventy-eight grape samples and samples of other host plants were collected and analysed: peach, plum (prune), oleander, rush broom, common olive and laurel. *Xylella fastidiosa* was not detected during the study of this pathogen. No false positive or non-specific reactions were found. Additional surveys of the territory of the Republic of Crimea are planned for 2019.

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Harper

### **Improvement of the sampling method for the monitoring of *Xylella fastidiosa* in Apulian olive groves**

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**Abstract:** An effective monitoring programme for *Xylella fastidiosa* relies on accurate sampling procedures in the pathogen-free areas of olive trees, as the primary host of the bacterium, to early detect the infection before symptoms develop. In this study, the current method of sampling plant material for *Xylella* was improved in two olive groves (one of Ogliarola and one of Leccino cvs) located in the northern part of the infected area of Apulia. Sampling was carried out on olive trees with no or low degree of symptomatology, investigating the spatial and quantitative distribution of the pathogen at canopy level throughout the year (from December 2017 to March 2019). After assessing the total infection rate in the first sampling, 10 trees per grove were selected based on the absence or presence of mild symptoms (8 ELISA-positive and 2 ELISA-negative) for a more in-depth and accurate sampling at two canopy levels (low and high), which was conducted at 6 times: December 2017 (T0), March 2018 (T1), June 2018 (T2), September 2018 (T3), December 2018 (T4) and March 2019 (T5). During the sampling periods, temperature ranges and averages were also recorded through a climatic station in the area. Two matrices, twigs and mature leaves were used for the analysis. The same portion of the two matrices was analysed by serological methods (ELISA) and qPCR as single or composite samples. The number of positive samples from the high level of the olive canopy throughout the period was significantly higher than those from the low level, regardless of the cvs. However, a significant increase in the detection of the pathogen in the lower part of the trees was found in the period June–September only in the cv Leccino. In the same period, however, the results of the qPCR showed a decrease in bacterial content. As for the matrices, the twigs were the best compared with the lower portion of the leaf with petiole. The results on the spatial and quantitative detection of *Xylella* in the canopy and in the two matrices for (T4) and T(5) is underway as the correlation between the detection of pathogens and temperatures (averages and ranges). In addition, the sampling schemes at grove level are also under evaluation.

## Official survey of *Xylella fastidiosa* in Croatia

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**Abstract:** *Xylella fastidiosa* is one of the most dangerous bacteria spread throughout the world and is one of the regular survey programmes within the EU. A special survey programme called '*Xylella fastidiosa* and vectors in Croatia' is being implemented in 2014. The official survey of *X. fastidiosa* strain CoDiRO includes visual examinations and sampling of symptomatic and asymptomatic plants. Sampling of potential vectors was also carried out. A major emphasis was given on plants susceptible to the attack of this bacterium and others that confirmed the infection in other countries where *X. fastidiosa* is present. It also included other hosts plants that can be found on Croatian territory, either domestic or imported plants. A large number of analysed samples referred to *Olea europea*, *Nerium oleander*, *Polygala myrtifolia*, *Prunus* sp., *P. dulcis*, *P. avium*, *P. cerasifera*, *Lavandula* sp. and others. Monitoring and analysis of the collected samples were carried out by the Plant Protection Centre and the Phytosanitary Inspection. The official survey programme includes olive-growing areas, orchards, urban zones, public areas, gardens and garden centres. The survey was carried out in six counties: Istria, Primorje – Gorski Kotar, Zadar, Šibenik – Knin, Split – Dalmatia and Dubrovnik – Neretva, while sampling of plant material from garden centres was carried out in 11 counties. The conducted laboratory analyses of the collected plant material as well as the captured potential vectors in the first place of the insect *Philaenus spumarius* confirmed that the disease of *X. fastidiosa* is not present in Croatia. It is important to continue to perform thorough surveys, since only early detection increases the effectiveness of any control measures as well as the prevention of further spread these bacteria within the EU.

## Specific PCR detection of *Pseudophaeomoniella* spp. into the xylem of healthy and *Xylella fastidiosa*-infected olive trees

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**Abstract:** Several fungal species have been found associated with the Olive Quick Decline Syndrome (OQDS) caused by *Xylella fastidiosa* subsp. *pauca* (Xfp) in Apulia, Southern Italy. The xylem-inhabiting species, *Pseudophaeomoniella oleae* and *Ps. oleicola*, were associated with brown or black wood streaking of various olive varieties, both on young and centenarian trees. However, specific pathogenicity tests conducted on olive plantlets indicated that these fungal species have a marginal role in the aetiology of OQDS. Considering the wide distribution of *Ps. oleae* and *Ps. oleicola* over the olive-growing areas in Apulia, a genomic characterisation study was started, in order to investigate the biology of these fungi, and to ascertain any possible interaction with Xfp and the OQDS. Several polymerase chain reaction (PCR) primers were designed from the internal transcribed spacer (ITS) regions of the rDNA genes of *Pseudophaeomoniella* spp. in order to develop a species-specific detection method. Primers were screened against the two reference strains, *Ps. oleae* FV84 and *Ps. oleicola* M24, and six more *Pseudophaeomoniella* spp. isolates, resulting in the amplification of a single specific amplicon from most of the primer pairs tested. Fifteen primer pairs confirmed their specificity when tested against several isolates of different xylem-inhabiting fungal genera, such as *Phaeoacremonium*, *Pleurostomophora*, *Phaeomoniella*, *Ochroconis*, *Paraconiothydium*, *Aspergillus*, *Lophiostoma* and *Cladosporium* spp. The size of the obtained amplicons enabled the use of some primer pairs in a Real-time PCR test, using a SYBR® Green format. Results confirmed the specificity of the tested primers, thus allowing the detection and quantification of *Ps. oleae* and *Ps. oleicola*, into the xylem of both healthy and Xfp-infected olive trees. Further research is in progress to develop a specific probe for qPCR quantification of the targeted fungal species in the olive plant.

## Molecular rapid detection of *Xylella fastidiosa*

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**Abstract:** An effective framework for early warning and rapid response is a crucial element to prevent or mitigate the impact of biological invasions of plant pathogens, especially at ports of entry. Molecular detection of pathogens by using PCR-based methods usually requires a well-equipped laboratory. Rapid detection tools that can be applied as point-of-care diagnostics are highly desirable, especially to intercept quarantine plant pathogens such as *Xylella fastidiosa*, one of the most devastating pathogens in crop and natural ecosystems. To this aim, in this study we developed a LAMP (Loop-mediated isothermal amplification) assay able to detect the target pathogen both in DNA extracted from axenic culture and in infected plant tissues. By using the portable instrument Genie® II, the LAMP assay was able to recognise *X. fastidiosa* DNA within 30 minutes of isothermal amplification reaction, with high levels of specificity and sensitivity. This new LAMP-based tool, allowing an on-site rapid detection of the bacterium, is especially suited to use at ports of entry, but it can also be profitably used to monitor and prevent the possible spread of the invasive pathogen in natural ecosystems.

## Situation of *Xylella fastidiosa* in the Alicante outbreak of Spain and phytosanitary measures adopted

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**Abstract:** In June 2017, *Xylella fastidiosa* was detected in a plot of almond trees showing typical symptoms of leaf scorch in the municipality of El Castell de Guadalest (Alicante, Comunidad Valenciana, Spain). It was the first detection of the bacterium in the Iberian Peninsula (mainland Spain). Immediately, the regional Plant Health Service adopted all the phytosanitary measures in accordance with Commission Implementing Decision 2015/789 of 18 May, to prevent the introduction and spread within the EU of *X. fastidiosa*. The current demarcated area (DA), that now covers 134,581 hectares, has been increasing since the first detection due to surveillance, sampling and molecular analysis activities. In fact, in the DA during 2017 and 2018 more than 18,000 samples from different plant species have been sampled during a seasonal period favourable for the detection of the bacterium. In 2017, 201 samples out of a total of 7,650 samples, all from almond trees, were positive for *X. fastidiosa*. In 2018, 11,069 samples were taken in the DA, and *X. fastidiosa* was detected in 1,157 of them, and not only in almond trees (95.6% of the positives) but also in other species: *Polygala myrtifolia* (1.4%), *Rosmarinus officinalis* (0.3%), *Helichrysum italicum* (0.9%), *Prunus domestica* (0.3%), *Prunus armeniaca* (0.7%), *Rhamnus alaternus* (0.3%), *Calicotome spinosa* (0.2%) and *Phagnalon saxatile* (0.3%). All of these new host species were found around infected almond trees. Up to now, all the MLST analysis performed on samples from more than 39 different municipalities in the DA, in almond and other hosts, showed that only subspecies *multiplex* and the same sequence type, ST-6, is present in the DA. In addition, the Plant Health Service have also carried out periodical samplings of potential vectors, and *X. fastidiosa* has been detected in specimens of both *Philaenus spumarius* and *Neophilaneus campestris*. All specimens analysed were infected by the subspecies *multiplex* and ST-6 as confirmed by MLST analysis. Eradication measures have been taken since the first detection, and so far more than 40,000 almond trees have been removed and shredded according to the Commission Decision.

## Public interest about *Xylella* in Italy, Europe and beyond – a spatio-temporal study

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**Abstract:** Google Trends is a freely available tool that makes it possible to monitor public interest in a topic, worldwide or for particular regions. Public interest is assessed on the basis of online behaviour, i.e. the number of Google queries for keywords representative of the topic of interest. This tool is increasingly used in public health as a web-based surveillance system for epidemics of human diseases. The assumption is that public interest is directly related to epidemic development. The tool (similarly to other web platforms such as Twitter and the Medisys media monitoring system) provides the potential to monitor the spatio-temporal development of public interest in plant diseases and, potentially, of plant disease epidemics too. This contribution uses Google Trends to reconstruct public interest in *Xylella fastidiosa* since 2004 in Italy, Europe and beyond. The data show the Italian, European and worldwide regions where people are most concerned by the epidemic. The tool thus provides a straightforward way for stakeholder mapping for a specific plant health threat. There is a good match between the timepoint of the peak in public interest for *Xylella* and the date of the first report of the pathogen in Italy, France, Spain and Portugal. There is thus the potential to use Google Trends for broad-scale surveillance purposes also in plant pathology. The tool also makes it possible to study public interest in various topics over time in a comparative way. For example, after the first finding of *Xylella* in Apulia in 2013, Google searches for the term 'Xylella' in Italy have become just as frequent as those for 'olive oil', whereas worldwide public interest in olive oil dwarfs that in *Xylella*. Taken together, these results suggest that web-based surveillance of emerging plant diseases could become another useful tool for plant health authorities, researchers and risk assessors.

## A combined analytical and hyperspectral approach for early detection of *Xylella fastidiosa* in olive plants: preliminary results

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**Abstract:** Among the several approaches to study the infection by *Xylella fastidiosa* (Xf) in olive plants, the combination of nuclear magnetic resonance (NMR), high resolution mass spectrometry (HRMS) and hyperspectral reflectance (HR) techniques is very promising as it provides a comprehensive view of the metabolome and the spectral range of infected olive plants. Indeed, NMR gives information on molecules with relatively high concentration (typically, primary metabolites), HRMS provides details on molecules at low concentration levels (typically, secondary metabolites) whereas HR offers valuable information on the biochemical and biophysical characteristics of the plant. Comparison of the metabolic and hyperspectral profiles of both infected and control plants can be exploited to identify markers of the infection and correlated wavelengths to set up analytical and hyperspectral strategies for early detection.

In this study our attention was devoted to the effects of infections by *Xylella fastidiosa* (ST53) in combination or not with fungi isolates correlated with the oOlive Quick Decline Syndrome on olive leaf composition. Different sets of infected olive leaves were submitted to investigation also taking into account simultaneous infections by fungi isolates of *Phaeoacremonium* (F1 (*Rubrigenum*) + F2 (*Aleophilum*)) and *Pseudophaeomoniella* (F3 (*oleae*) + F4 (*oleicola*) + F5 (*oleicola*)).

NMR, HRMS and HR data of control and infected leaves were submitted to Principal Component Analysis (PCA) to perform the following comparisons: Xf vs Control; Xf vs (XfF1 and XfF2); Xf vs (XfF3, XfF4 and XfF5). NMR, HRMS and HR results indicated that, even though fungi infections are operative, *X. fastidiosa* causes peculiar changes of the metabolic and spectral profiles. In fact, Xf-infected samples are differentiated from those not infected by Xf. Metabolites mainly related to the infections by *X. fastidiosa* are Oleuropein derivatives.

The study is ongoing on the correlation of HR with HRMS and NMR specific statistical analysis.

## A new device for rapid and on-site pathogen detection

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**Abstract:** A prototype of a diagnostic device for molecular analysis has been designed, developed and realised. The device is able to fulfil every step needed to perform isothermal gene amplification: DNA extraction, amplification and real-time revelation through fluorescence analysis. The excitation and emission wavelengths are respectively 470 nm and 530 nm. With this device it's possible to perform 48 tests at the same time using standard 0.2 ml PCR tubes; specifically four different analyses can be performed at the same time, in 12 test groups each. The analysis duration depends on the specific analysis to be performed; it is generally between 30 and 60 minutes. To trace all performed experiments, a technology for operator and experiment identification has been integrated in the system; all information is recorded locally and in the cloud, allowing remote access through a safe and encrypted system. The extraction and amplification temperatures handled by the device are between 55 and 90 °C, with 0.1 °C accuracy. The device handling interface has been realised with an Android application on a tablet connected to the device via bluetooth; through the interface it's possible to:

- automatically identify the user, through the use of RFID badges/bracelets

- recognise the test type, through the use of microchips embedded in the kits
- recognise the sample that has to be analysed, through the use of printed or RFID labels or through the import of pre-loaded data from the server
- visualise real-time plots with the results of the analysis and experiment reports with data interpretation.

The device has been designed to have a small size to allow on-site direct use, through an (external) battery or through standard 12 V car plugs.

### **Absence of *Xylella fastidiosa* in Tunisia: preliminary survey results**

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**Abstract:** Tunisia is one of many olive-producing countries, with almost 2 million acres of olive plantations. *Xylella fastidiosa* is a xylem-inhabiting pathogen which can infect more than 580 plant species, although most host species are symptomless. This pathogen was primarily limited to North and South America, but in 2013 a widespread epidemic of new a disease called 'Olive Quick Decline Syndrome' caused by this fastidious pathogen appeared in south-eastern Italy, and later in other European countries (France, Germany and Spain). Since then, this pathogen has become a serious threat to Tunisian flora. The national phytosanitary authorities have adopted several measures to prevent the introduction of *X. fastidiosa* into the national territory by restricting the importation of host plant species from infected areas and conducting regular field surveys. The project starts with orchard surveys and sampling from olive trees and seedlings during the period of February 2015 to June 2018. Samples were analysed by conventional PCR using two sets of primers (EPPO 2016). The results of PCR amplification form the total DNA isolation from plant tissues showed negative results in comparison to the positive control. These preliminary results indicate absence of *X. fastidiosa* in Tunisia. Larger scale, sampling of other plant hosts growing near to olive orchards and insect trapping will be conducted to ensure its absence in Tunisia.

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EPPO, 2016

### **Austrian *Xylella fastidiosa* monitoring in the context of EUPHRESKO projects: basis for risk assessment driven by climate change?**

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**Abstract:** Since 2013, when *Xylella fastidiosa* (Xf) was first detected in olive trees in Southern Italy, a considerable number of new interceptions and outbreaks were recorded in several European countries, *inter alia*, several interceptions on coffee plants in Austria. The entry pathways and the means of spread are still not fully understood. However, the strongest evidence for the pathogen's movement and dispersal is the trading of infected host plants for planting and infectious insect vectors.

For a better assessment of the prevalence of Xf in plant trade, the Austrian activities within EUPHRESKO research projects focused on the testing of high-risk plants and grapevine planting material in garden centres and nurseries. Sampling and testing of symptomatic host plants in different crops and differentiation from look-a-like symptoms such as 'Roter Brenner' (*Pseudopeziza tracheiphila*) in grapevine was carried out. In addition, surveys on the occurrence, seasonal abundance and epidemiologic characteristics of potential Xf vectors in vineyards and *Prunus* orchards are being carried out and will be enlarged to an olive grove in the eastern part of Austria.

The methods used, preliminary results of the vector species composition and abundance together with the outcome of the Austrian surveillance on traded plants and planting material will be presented.

Finally, the possible consequences of climate change for the establishment and spread of Xf in Austria will be discussed. This will result in a risk mapping for Austria, based on field experiments with Pierce's disease conducted by US experts from the University of California Berkeley (Cooperative Extension) where Xf has been present for several years.

## BRIGIT Vector-Borne Disease of Plants

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**Abstract:** *Xylella fastidiosa* is not present in the UK and preventing its establishment is a priority. BRIGIT is a UK Research and Innovation funded, two-year project, focusing on enhancing surveillance and UK preparedness for *X. fastidiosa*. The project, a partnership between 10 research organisations, has four work packages focusing on: citizen science, outreach and knowledge exchange; enhancing diagnostic capabilities; investigating insect vector biology; and epidemiology and modelling of disease spread.

Forest Research, the Royal Horticultural Society and the University of Sussex are working together to deliver the first aim, focusing on: trees, ornamental plants and insect vectors, respectively. The main outcome from this work package is to engage with the public, policymakers and key stakeholders so they can participate in knowledge exchange to reduce the likelihood of *X. fastidiosa* entering the UK but also help mitigate the impact should it do so.

We shall deliver this goal through: generating informative websites and databases; training sets of volunteers to be expert spotters of *X. fastidiosa* hosts, disease symptoms and insect vectors; and public engagement activities. Engaging with citizens will lead to increased surveillance for *X. fastidiosa* and increase our current knowledge of insect vector and host distribution.

## Comparison of real-time PCR protocols for detection of *Xylella fastidiosa* in different plant species and cultivars

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnte 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** Detection is one of the first steps to prevent the introduction and spread of *Xylella fastidiosa*. This task can be influenced by the bacterial concentration and the type of plant species analysed. The diagnostic standard for *X. fastidiosa* EPPO 2018 PM 7/24 (3) includes several protocols of real-time PCR addressed to different genome targets. In order to determine the accuracy of these PCR protocols, samples of healthy plant material from five cultivars of almond (Marcona, Marta, Avijor, Belona, Constantí) and eight of olive trees (Alfara, Arbequina, Blanqueta, Cornicabra, Frantoio, Hojiblanca, Manzanilla, Picual) were spiked with tenfold serial dilutions of the strain IVIA 5901 of *X. fastidiosa* subsp. *multiplex* ST6 isolated in the outbreak in Alicante (Spain). Spiked samples of other plant species such as grapevine, citrus, oleander, pistachio, loquat and persimmon are also being challenged. In all cases, the CTAB method was used for DNA extraction. Sensitivity is being analysed between PCR protocols for the same cultivar, as well as between species or cultivars for the same real-time PCR, and so far slight differences have been found. Moreover, these protocols are being assayed with samples of almond trees from the infected area in Alicante. In some cases, problems of inhibition have been detected with certain real-time PCR protocols. All these results can give clues to optimise the detection

protocol depending on the host and the specific situation of the area of origin of the plant material analysed.

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### **Current situation after *Xylella fastidiosa* first outbreak in an olive grove in mainland Spain**

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**Abstract:** A first case of infection caused by *Xylella fastidiosa* subsp. *multiplex* ST6 was detected in an olive tree in the municipality of Villarejo de Salvanés, in the southeast of the Community of Madrid (Spain) in April 2018. This constituted the first detection of *X. fastidiosa* in this crop on the Iberian Peninsula, and the second concerning other crops after the detection of the pathogen in almond trees in Alicante, in the Valencian Community (Spain), where *X. fastidiosa* subsp. *multiplex* ST6 was also identified, although until now it has not been detected causing infection in olive trees in that region.

The Community of Madrid accounts for 27,000 hectares of olive trees, most of them in Villarejo de Salvanés. This area has a continental climate, with below zero minimum winter temperatures. After the official declaration of the outbreak, the actions established in the European Decision 2017/2352, the Contingency Plan against *X. fastidiosa* of the Spanish Ministry of Agriculture, Fisheries and Food, and that of the Community of Madrid were immediately applied to eradicate the bacterium and prevent its spread.

Among these measures, around 2,000 samples from olive trees and other host plants of the demarcated area (DA), 300 insect vectors, and olive trees from other municipalities were analysed. All of them gave a negative result by real-time PCR official protocols. As a direct consequence of the application of the eradication measures, the results obtained so far would indicate that this detection was a unique event and the bacterium would be neither disseminated nor established in the EU, although surveillance measures within the DA must continue as established in the Action Plan of the Community of Madrid.

### **Detection and identification of *Xylella fastidiosa* in France: improvement of the detection scheme**

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** *Xylella fastidiosa* was identified in France in 2015, first on Corsica, then in the Provence-Alpes-Côte d'Azur administrative region (French Riviera). Forty-nine host plants have so far been identified, infected by *X. fastidiosa* subsp. *multiplex* belonging to the sequence type ST6 or ST7. Also in these two areas, the vector *Philaeenus spumarius* has been found positive for these two sequence types.

In France, the detection scheme for plants and vectors is based on the real-time PCR Harper et al. (2010) after a high-throughput DNA extraction based on the QuickPick™ Plant DNA kit used with a KingFisher™ robot. This method was evaluated in 2014–2015, showing excellent performance criteria, apart from a lesser sensibility on olive tree (*Olea europaea*) and some oak species (*Quercus* spp.). In order to improve the limit of detection on these matrices, collaborative works with INRA Angers led to the evaluation of a new protocol based on sample sonication, in order to break *X. fastidiosa* biofilms, CTAB DNA extraction and modified parameters for the real-time PCR mix. Although this method is time consuming, it allows a real improvement of the limit of detection for olive tree and holm oak despite the presence of PCR inhibitors.

Strain identification is performed using MLST scheme (www.pubmlst.org) with the PCR Yuan et al. (2010) for amplifying sequences of 7 housekeeping genes. On plant DNA extracts giving high Ct values with the real-time PCR Harper et al. (2010), some amplification failures were observed. A protocol with addition of BSA into the PCR reaction mixture has been validated, showing improved performance in term of sensibility allowing success for strain typing even in the presence of a small amount of target DNA.

### **Different approaches for detection of *Xylella fastidiosa* by molecular techniques**

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** The outbreak of *Xylella fastidiosa* in several European countries on many plant hosts make it necessary to have fast, sensitive, and specific methods that allow large-scale surveys in wide areas, in order to know the degree of dispersion of the pathogen. For this purpose, molecular techniques that do or don't require DNA purification have been developed in diagnostic kits by different companies. In this work, a comparative study has been made of some of these kits analyzing a set of infected almond tree samples from the demarcated area of the outbreak in Alicante (mainland Spain). The real-time PCR by Harper et al. (2010, erratum 2013) and Francis et al. (2006) after manual DNA purification by CTAB was considered the gold standard protocol. First, four different master mixes for real-time PCR were tested, and one of them was selected. Then, the methodologies to be challenged were the automatized DNA extraction with Maxwell® RSC PureFood GMO and Authentication Kit in a Maxwell® RSC instrument (Promega), the *Xylella* Screen Glow kit based on LAMP technology with the ICGENE system (Enbitech), the AmplifyRP® XRT+ Isothermal Amplification kit based on recombinase polymerase amplification (Agdia) and phyAlert® kit based on a triplex PCR (MICROGAIA BIOTECH). Interestingly, DNA measurements were not informative of the sensitivity of the protocols challenged.

Similar qualitative results were obtained with all the protocols, each of them with advantages and disadvantages. The choice can be based on the sample size and the economic and human resources of the laboratory. In general, a test with no DNA purification could be used in infected zones for a first screening, further analysing the negative samples with DNA purification previous to real-time PCRs recommended in EPPO 2018 PM 7/24 (3).

### **Improving *Xylella* sampling in Mallorca**

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**Abstract:** *Xylella fastidiosa* was detected in Majorca in late 2016 (Olmo et al. 2017). Since then, accomplishing EU regulatory, a huge number of samples have been analysed.

Particularly, the Mallorca outbreak is different to others in Europe, because of the coexistence of subspecies *multiplex* (ST81, ST7) in almond trees, olive trees and other species, and subspecies *fastidiosa* (ST1) in almond trees and grapevine plants among others.

As already known, detection tests of *X. fastidiosa* are conditioned by the sampling date. However, it is not well known if the optimal dates for sampling are the same for all host species, if it is viable to pool several samples, or if the bacterium can be detected in old wood samples.

In this study, we have focused the *X. fastidiosa* monitoring on almond, grapevines and olive trees. Monthly percentages of positives and average Ct value of more than 2,000 analyses of each of these crops were compared. Overall, we observed that for almond trees the best results were obtained from samples collected from June to August, whereas in grapevine the optimum period was from August to October. In olive trees early spring resulted in the most favourable time for detection, with an increase in Ct and decrease of positive cases in summer.

In the assays conducted with pooled almond leaf samples, mixing one infected plant extract with the same volume of up to four negative extracts, showed an average Ct increase of 2.7 cycles, whereas for olive and grapevine samples the Ct increase was > 4 cycles.

By default, *X. fastidiosa* is normally analysed in leaf midribs and petioles. We also tested the potential use of wood samples from trunks, sampling different tree rings. Although this sampling procedure is not recommended for routine surveys, it allowed us to establish a potential infection chronology of the number of years since the first infection might have occurred in the Balearic Islands.

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### **Risk-based delimiting survey strategy for *Xylella fastidiosa***

Diakaki M\*, Camilleri M, Cortiñas J, Schenk M, Zancanaro G, Vos S

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**Abstract:** This poster was prepared in the context of the EFSA mandate on plant pest surveillance (M-2017-0137), at the request of the European Commission. It provides guidance on the steps of a delimiting survey which are to be followed, upon a new positive finding during a detection survey of *Xylella fastidiosa*. The main objective of a delimiting survey is to establish the boundaries of an area considered to be infested by or free from the pest. The proposed approach is based on a sequence of sampling rounds starting from the periphery, at a certain distance from the centroid of the area to delimit and going towards that centroid. This distance should be based on relevant biological information such as pest spread capacity. Insect vector and plant material should be sampled in the context of the EFSA delimiting survey approach. For both vectors and plants, the sample size is calculated in order for surveillance to achieve an overall 95% confidence level with a 0.5% design prevalence in detecting *X. fastidiosa*. This is done using the statistical tool RiBESS+ which is available online with open access after registration. Following the proposed step-wise approach, an infected zone and corresponding buffer zone can be established.

### **Risk-based detection survey strategy for *Xylella fastidiosa***

Diakaki M\*, Camilleri M, Cortiñas J, Schenk M, Zancanaro G, Vos S

\*Animal and Plant Health Unit (ALPHA), European Food Safety Authority (EFSA)

**Abstract:** This poster was prepared in the context of the EFSA mandate on plant pest surveillance (M-2017-0137), at the request of the European Commission. Its purpose is to describe in details the main parameters necessary for a statistically sound detection survey for *Xylella fastidiosa*. In addition, the risk factors relevant for this pest and their relative

risks are presented. The risk-based detection survey is to be conducted on a yearly basis to substantiate pest freedom. Its main objective is to ensure detection if the pest is present. Surveillance activities initiated with visual examinations in the field which are followed by field sample selection and diagnostic laboratory testing. Visual examination is ineffective on asymptomatic plant material and, thus, sampling and testing are always required. Insect vector and plant material should be sampled in the context of the EFSA detection survey approach. For both vectors and plants, the sample size is calculated in order for surveillance to achieve an overall 95% confidence level with a 1% design prevalence in detecting *X. fastidiosa*. This is done using the statistical tool RiBESS+ which is available online with open access after registration. Following the proposed detection survey approach, a statement of pest freedom can be substantiated.

### **Supporting early detection of *Xylella fastidiosa* by using 'indicator plants' and improved molecular detections assays**

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**Abstract:** Early detection of *Xylella fastidiosa* (Xf) in new areas constitutes a factor of fundamental importance for a successful eradication strategy of this pathogen (1). To this end, in the frame of the European project 'XF-ACTORS', the Laboratory of Bacteriology of Benaki Phytopathological Institute (LB-BPI) is participating in a research task of this project to evaluate the use of selected plant species (2), exhibiting rapidly characteristic symptoms upon Xf infection, as 'indicator plants' in risky locations to support early detection of Xf. In this connection, LB-BPI is using such indicator plants exposed in selected high-risk areas in Greece, i.e. two harbours, an airport and an open market where high-volume trade of plants for planting occurs. The indicator plants have been placed in pots in the selected locations and are monitored and tested regularly according to EPPO-recommended methods (conventional PCR and qPCR) (3). So far, none of the indicator plants has been found infected by Xf. Additionally, research is being carried out at LB-BPI to improve the sensitivity of the PCR-based methods by treating the plant DNA extracts before amplification with a commercially available mixture of polymeric materials to segregate PCR inhibitors (4). Moreover, in a parallel project of the LB-BPI, the possible interference in the Xf detection assays of the microbiome associated with Greek olive varieties is being examined to obtain relevant data to further support optimisation of the respective diagnostic protocols.

### **Survey for the presence of *Xylella fastidiosa* on olive plantations in the Siwa region, Egypt**

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**Abstract:** Olive (*Olea europea*) is a common crop cultivated in many Mediterranean countries as well as in Egypt. The crop is currently threatened by the devastating pathogen; *Xylella fastidiosa* in many countries worldwide. The bacterium *X. fastidiosa* is gram-negative, xylem-inhabiting and causes numerous diseases in more than 360 hosts. The recent finding of the pathogen to induce the Olive Quick decline Syndrome in Italy has led many countries to conduct mandatory survey programmes to investigate its presence in various host plants. During summer 2018, a survey was conducted in Siwa Oasis, Western desert of Egypt. Several trees displaying leaf scorching, dieback and decline symptoms, resembling those associated with *X. fastidiosa* were observed. A total of 100 samples were collected from five major areas (Siwa, Aghormy, AbouElShourof, Bahii ElDeen, and Elmarak). The collected samples belonged to 10 different olive cultivars and the age of



trees varied from 6 up to 50 years old. Sampling density was planned according to surface area of olive cultivation in each area, so as to have a sampling density of one sample per 120–180 Feddan. Also, visual inspection was planned to cover 30 trees per sampling unit. Priority was made for sampling from trees showing leaf and branch drying symptoms. All the samples were tested for the presence of *X. fastidiosa* by Loop mediated isothermal amplification (LAMP) technique following the EPPO protocol and manufacturer's manual (ICGENE, Enbiotech, Italy). The results showed that all the tested samples were negative for the presence of *X. fastidiosa*. The samples were also analysed for other biotic infection and abiotic factors. The symptoms were found to be associated with root rot pathogens such as *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani* and with *Meloidogyne* spp. The chemical analysis of water irrigation and soil samples showed extremely high salinity (6000–8000 siemen/metre) which can be the major stress on olive trees in Siwa. This preliminary survey concluded that olive plantations in Siwa are still free of *X. Fastidiosa*; however, more future survey programmes are needed in this region and others in Egypt.

### Survey for *Xylella fastidiosa* and xylem-sap-feeders in Lebanon

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**Abstract:** The emergence of *Xylella fastidiosa* in Europe and the report of some suspicious leaf scorching symptoms in Lebanon affecting oleander and olives prompted for further targeted surveys. Field inspections and sampling covered the main fruit tree growing areas in Lebanon (Bekaa valley, South Lebanon and North Lebanon). In summer 2018, more than 500 plant samples were collected from trees showing suspicious leaf symptoms. The majority consisted of olives (*n.* 298) followed by grapevine (*n.* 96), citrus (*n.* 72), stone and pome fruits, and walnut. Mature leaves from all samples were firstly screened by ELISA and then a group of 100 randomly selected and re-tested by PCR following the procedures recommended in EPPO diagnostic standard 7/24 (3). None of the samples tested yielded positive reactions either in ELISA or PCR tests. Surveys to characterise the entomofauna occurring in the olive orchards and vineyards (mainly in Northern Lebanon, Bekaa valley and Jezzin region) were periodically carried out from spring to summer 2018, collecting insects from the canopies of the trees or from the weeds or border plants by sweeping net. Based on the morphological identification, among the specimens collected none were xylem-sap-feeders while several species of leafhopper (Hemiptera: Cicadellidae) and planthopper (Hemiptera: Fulgoroidea) were identified. Field surveys and inspections at the port of entry are in place to prevent inadvertent introduction of plant propagating materials infected by *X. fastidiosa*.

### XylAppEU\_2.1.3 for precise acquisition and traceability of monitoring data of *Xylella fastidiosa* in the EU

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**Abstract:** XylAppEU 2.1.3 will be launched at the end of 2019 as the final version of the Android application XylApp\_1.2.4 used for the official monitoring of *Xylella fastidiosa* in Apulia, Italy. It was developed on the basis of the characteristics and input received from stakeholders in three EU pilot areas (Córdoba, Spain; Crete, Greece; Apulia, Italy) and from other project partners. A video tutorial of the previous version (2.1.2) was prepared and launched on the project website and the application download was available for testing between partners. This version considers the following aspects which will be implemented with additional input from partners after testing: (i) analysis of the scenario in the specific

country; (ii) identification of the specific needs and resolution conditions in relation to the different users; (iii) definition of the featured applications, constraints, performance, interfaces and any other features to fulfil users' needs; (iv) active engagement of end-users for the finalisation and validation of the app.

The advanced application XylAppEU\_2.1.3 was equipped with grids of different sizes (e.g. 100 m x 100 m, 1 km x 1 km) of the three pilot sites, based on the free formats available from the European Environment Agency (EEA). With regard to the hardware and other minor functions, different parameters (sensor configuration, connectivity and size of the display) were implemented for the most popular platforms (e.g. Google Android). The following specific features were also implemented: transmission protocols for data sending over the internet; inclusion of grid visualisation of the pilot sites for sample localisation at EU level (ETRS89-LAEA Europe); development of a GIS model for management of EU cartographic data. A new graphic interface has been made, user friendly, which guarantees better functionality and integration of modules, beside guidelines or better management by users. This version allows the acquisition, storage and transmission of data in different formats. As for the previous version, a video tutorial on XylAppEU\_2.1.3 will be prepared for dissemination purposes.

### ***Xylella fastidiosa* does not occur in Moroccan olive groves**

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**Abstract:** With an area of cultivation of about 1.01 million ha, olive (*Olea europaea* L.) ranks first among cultivated crops in Morocco and represents one of the most important crops for the country's economy. In October 2013, an outbreak of *Xylella fastidiosa* was found in Apulia (south-eastern Italy) in olive trees affected by olive quick decline syndrome, and later several *X. fastidiosa* outbreaks have been reported in other European countries (France, Germany, Spain and Portugal). The world map of the Köppen-Geiger climate classification shows a similarity between the climates of the affected areas in the Europe and those of Morocco. Consequently, the Moroccan climate might not be a limiting factor for the establishment of *X. fastidiosa*, thus representing a serious threat to the Moroccan olive industry. During 2015–2018, surveys were conducted on *X. fastidiosa* in several olive groves. In a few trees, severe symptoms which could be associated to the bacterium were observed. A total of 720 olive trees (cv. Picholine Marocaine) were sampled from 30 different commercial groves in two regions (Azilal and Meknès). ELISA and PCR were used to check for the presence of the bacterium; results obtained did not show any positive sample. These preliminary results indicate the absence of *X. fastidiosa* in Moroccan olive groves. Furthermore, more scale and continuous surveys are needed to ensure its absence in the country.

### **Harmonisation of laboratory diagnosis of *Xylella fastidiosa* among national reference laboratories**

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnte 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** On 27 March 2019, the European Commission designated the European Union Reference Laboratory (EURL) for Bacteria in Plants that will operate within the framework of official controls in plant health. The EURL for Bacteria in Plants is a consortium consisting of four partners, namely: 1. the Research Centre for Plant Protection and Certification CREA-DC (Italy); 2. the Research Institute for Agriculture, Fisheries and Food ILVO (Belgium); 3. the National Institute of Biology NIB (Slovenia); and 4. the National Reference Centre NRC-NVWA (the Netherlands). The major objective of the EURL for

Bacteria in Plants is to support the activities of the Commission in relation to risk assessment and risk management of plant pests, to facilitate and harmonise laboratory diagnosis and analyses and to coordinate activities of the corresponding National Reference Laboratories (NRLs).

The EURL for Bacteria in Plants aims to achieve an overall high level of diagnostics at National Reference Laboratories (NRLs). Among the organisms listed as Union quarantine pests, emphasis on the priority pest *Xylella fastidiosa* has been given for the period 2019–2020. Based on the consolidated Work Programme of the EURL for Bacteria in Plants, details and guidance on internationally recognised (e.g. EPPO diagnostic standard PM 7/24) test protocols for the detection of *X. fastidiosa* will become available to all NRLs. A PT will be organised in 2019 to assess the diagnostic competence of laboratories to detect *X. fastidiosa* in selected host plants. A questionnaire, prior to the PT, will collect information on the technical aspects of test protocols currently used by the NRLs.

### **Comparison of real-time PCR and droplet digital PCR for the detection of *Xylella fastidiosa* in plants**

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**Abstract:** *Xylella fastidiosa* (Xf) is a quarantine plant pathogen bacterium originating from the Americas and that has emerged in Europe in 2013. Limited to the xylem vessels of plants, Xf has a broad host range estimated to encompass more than 560 plant species. Xf can be detected directly on plant macerate using molecular methods such as real-time PCR, which is a sensitive technique. However, some plants may contain components that can act as PCR reaction inhibitors, which can lead to false negative results or an underestimation of the bacterial concentration present in the analysed plant sample. Droplet digital PCR (ddPCR) is an innovative tool based on the partitioning of the PCR reagents and the DNA sample into thousands of droplets, allowing the quantification of the absolute number of target DNA molecules present in a reaction mixture, or an increase of the detection sensitivity. To improve Xf detection, the Harper et al. (2010) real-time PCR protocol, already used in the framework of surveys in several European countries, has been transferred to an optimised ddPCR protocol. This new assay was evaluated and compared with the initial real-time PCR on five plant matrices (*Lavandula angustifolia*, *Olea europaea*, *Polygala myrtifolia*, *Quercus ilex* and *Rosmarinus officinalis*) artificially inoculated and on naturally infected samples. This ddPCR assay enabled the detection of Xf in the five plant matrices artificially inoculated with a similar limit of detection, or a slight benefit for *Quercus ilex*, compared with real-time PCR. Moreover, ddPCR improved diagnostic sensitivity as it enabled detection of Xf in samples of *Polygala myrtifolia* or *Q. ilex* that were categorised as negative or close to the limit of detection. This makes this ddPCR protocol the first one that can be used for the detection of Xf.

#### *Bibliography*

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### **Implementation and validation of rapid diagnostic procedures for *Xylella fastidiosa***

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** Surveys for *Xylella fastidiosa* are now mandatory in the EU Member States and genetically diverse strains, i.e. those capable of infecting different hosts, have been intercepted in the currently known EU outbreaks and/or containment areas. Given the long list of the EU susceptible host plants, currently comprising more than 50 plant species, there is an impellent need to develop robust and rapid diagnostic tests (ready-to-use and on-site tests) suitable for testing and screening different plant matrices and large numbers of samples. To this end, we have compared membrane-capture-based methods and ready-to-use kits, the latter based on isothermal nucleic acid amplification techniques. Indeed, a chloroform-free method based on the use of the Maxwell® RSC PureFood GMO and Authentication Kit (Promega) was evaluated on different plant matrices, as an alternative protocol for recovering total DNA suitable for identifying *X. fastidiosa* in real-time PCR (qPCR) reactions, so far the most widely used diagnostic method. In a first validation test, 90 olive field-trees were tested simultaneously using different procedures: FTA-ELISA, DTBIA, qPCR assay on membrane-captured DNA, loop-mediated isothermal amplification/LAMP on fresh sap or on stored tissue-imprinted membranes, and recombinase polymerase amplification/RPA. Performance criteria for each test were determined by comparing the results with those obtained in qPCR assays performed according to Harper et al. (2010). The results, while suggesting that different types of membranes can be used for capturing bacterial cells for a subsequent serological or molecular detection, showed that all these approaches produced lower accuracy values than LAMP, RPA and standard qPCR. The chloroform-free DNA purification kit herein tested allowed to recover DNA templates of high quality with standardised yields, suitable for the detection of the bacterium using the qPCR protocols currently validated for *X. fastidiosa*.

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#### Bibliography

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### ***Xylella fastidiosa* is not detected yet in Jordan**

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**Abstract:** The aim of this project is to plan strong strategies for restriction the entrance of *X. fastidiosa* to Jordan, and ensure its absence in Jordan. The project starts with surveying orchards for the source of infection in potential hosts and in the imported and locally produced propagation plant materials. Samples from host trees and seedlings were collected in the period of February 2016 to March 2019: olive (950 samples), grapevine (198), stone fruits (157), pome fruits (15), citrus fruits(52) oleander (41), rosemary (105) ornamental plants (4), walnut (2), guava (2), xylem-feeding insects (10) and different plant materials imported to Jordan (146) were analysed by serological techniques (ELISA) using *X. fastidiosa*-specific antibodies and by conventional PCR using three sets of primers (EPPO 2016). The results of the Elisa assay on plant saps and PCR amplification from gDNA extracted from plant materials showed negative results in comparison with the positive control. These preliminary results indicate that *X. fastidiosa* is not yet detected in Jordan. Periodic surveys will be conducted to ensure its absence in Jordan and restricted quarantine regulation will be followed to prevent its entrance to Jordan.

### **Media and scientific literature monitoring on *Xylella fastidiosa***

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**Abstract:** Since 2017, EFSA has been carrying out the Horizon scanning project, set up in response to a long-term mandate of the European Commission. The objective of this project is to identify emerging plant health risks through media and scientific literature monitoring. In this activity, EFSA relies on the technical support of the Text and Data Mining Unit of the Joint Research Centre of the European Commission, the team behind the MEDISYS platform (a fully automatic event-based surveillance system). The ontology used (pest scientific and common names) covers around 800 pests, 400 of which are listed in the EU legislation or in the EPPO lists. Specific keywords also enable the screening of sources for news on not-listed pests of potential interest for the EU. Currently around 27,800 feeds corresponding to 10,400 sources of general news at national, regional and local levels from 202 countries are monitored. The platform also performs the monitoring of 2,000 scientific or specialised sources in fields related to plant production and plant health. *Xylella fastidiosa* is defined in the platform by 30 keywords corresponding to scientific and common names of the causal agents as well as of the disease. In the period February 2017–September 2019, around 16,700 articles from 96 countries were retrieved by the platform. Statistics and visual representation of the results of the three-year monitoring are presented. They show the association of trends and peaks to specific events in the countries such as the detection of new findings or outbreaks, and the implementation of mandatory control measures. The scientific literature monitoring is performed since December 2018. The topics mainly include early, rapid, and portable detection methods and vector biology.

## Session: Ecology, epidemiology and modelling

### Evaluating the efficiency of control strategies against *Xylella fastidiosa*; insights from the epidemiology of Pierce's disease

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**Abstract:** *Xylella fastidiosa* subsp. *fastidiosa* is the causal agent of Pierce's disease of grapevines. Pierce's disease has presented a serious challenge for the grapevine industry in the United States and turned into an epidemic in southern California due to the invasion of the insect vector *Homalodisca vitripennis*. In an attempt to minimise the effects of *X. fastidiosa* subsp. *fastidiosa* in vineyards, various studies have been developing intervention strategies to prevent the occurrence of Pierce's disease, i.e. prophylactic strategies. Moreover, even if there are no currently available strategies to cure infected vines, some strategies have been reported promising. We collected all these promising curative and prophylactic strategies and assessed them via an epidemiological model of *X. fastidiosa* subsp. *fastidiosa* that we developed. Based on the results of our analyses we could propose which types of strategies should be prioritised in the future to prevent the spread and establishment of *X. fastidiosa* subsp. *fastidiosa*. Our model's framework could offer considerable inspiration for the design of an epidemiological model of olive quick decline syndrome. Such a model could further help to pinpoint existing knowledge gaps and decide on the right control measures against *X. fastidiosa* in Europe.

### Modelling the spread and control of *Xylella* in novel outbreak locations

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**Abstract:** Predicting the spread of potential *Xylella fastidiosa* outbreaks in novel locations is of general interest in pest risk assessment throughout Europe. However, translating our knowledge of the disease to novel locations can be problematic due to the potential differences in environment and epidemiology. In turn, it is unclear how these differences affect the effectiveness of prescribed containment and eradication strategies. To address

this question, we present a model which simulates spread by coupling a generic epidemiological model with a dispersal–kernel model. Available data on the epidemiology and spread of *X. fastidiosa* in Apulia, Italy, are used to parameterise the model but the influence of changing the epidemiological and landscape parameters is also assessed. The model illustrates the effectiveness of the current measures in limiting further spread, and in some cases, reversing the expansion of infected areas and even eradicating *X. fastidiosa* outbreaks. Reducing buffer zone width in both containment and eradication scenarios increased the area infected. The importance of early detection of new outbreaks is key to successful control, demonstrating the importance of increased surveillance and detection capabilities. In addition, we present our preliminary results on the potential spread and control of *X. fastidiosa* outbreaks in the UK.

### **Risk-based surveillance strategies for early detection of *Xylella fastidiosa* in continental France**

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**Abstract:** *Xylella fastidiosa* has only recently been detected in metropolitan France, specifically in the southern region of Provence-Alps-Côte d'Azur. The threat posed by the potential spread of the bacterium to other parts of the country pushed national plant protection agencies to implement a monitoring campaign across the entire territory, with a special focus on those areas where the disease had already been detected. However, the sampling and monitoring effort that is still necessary to assess the actual dissemination of the bacteria is high, with many parts of the country still poorly sampled. In this work, we propose different surveillance strategies that aim at choosing the most effective areas where sampling and monitoring should be performed in order to detect the presence or arrival of the pathogen. These strategies are based on detailed risk maps of potential infection that have already been produced by the authors in another contribution. Our methodology involves the use of simulated epidemics on a spatial network representing the entire French territory at a fine spatial resolution and estimates the appropriateness of the different strategies according to different measures of efficiency in early detection. Finally, we will test different scenarios according to increasing sampling efforts that the surveillance agency may be willing to make and we will measure the marginal gain in detection for every added unit of sampling.

### **Bayesian analysis of climatic and spatial factors on *Xylella fastidiosa* distribution in the demarcated area in Alicante (Spain)**

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnte 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** Almond leaf scorch, caused by *Xylella fastidiosa* subsp. *multiplex* (Xfm), was detected in 2017 in Alicante (Spain). Since then, intensive surveillance has been carried out in the demarcated area. In this study, the effect of climatic and spatial factors in the geographic distribution of the pathogen was analysed. In addition, it was studied whether the risk levels based on the minimum winter temperatures, defined by A. H. Purcell for *X. fastidiosa* subsp. *fastidiosa* in grapevine in North America, can be extrapolated to Xfm in almond in Alicante. Average monthly climate data for 1970–2000 was obtained from the WorldClim version 2 database. Presence/absence data of Xfm in the official surveys in the demarcated area in Alicante in 2017 were analysed using a Bayesian approach, through the Integrated Nested Laplace Approximation (INLA). An advantage of Bayesian hierarchical spatial models is that they allow taking into account the spatial autocorrelation existing between the geographical locations of the observations while providing a more

realistic and accurate estimation of uncertainty. This spatial effect was included through a conditional autoregressive structure (iCAR). Climatic factors were not relevant in the model, likely due to the reduced geographic extent of the study area and the resulting limited variability of climatic covariates. Nevertheless, Xfm was detected within all minimum winter temperature thresholds defined by A. H. Purcell (from  $< 1.1$  °C to  $> 4.5$  °C), illustrating the known climatic adaptability of Xfm. A strong effect of the spatial component was obtained in the models. That is, the disease spread was largely defined by the spatial relationship among geographic locations.

### **Integrating spread modelling and remote sensing imagery to optimise early detection and spatial distribution estimation of *Xylella fastidiosa***

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**Abstract:** The long list of potential host species for *Xylella fastidiosa* (Xf) and its more recent detections in France and Spain demonstrate the potential for further pathogen spread throughout Europe, which has highlighted the importance of surveillance for protection of host plants against this pathogen. Xf is difficult to manage once it has become established in a territory, so effective surveillance efforts must be focused on the early detection for its eradication in new areas. However, the detection of early infections or even the spread of an establishing epidemic are limited by the cryptic nature of Xf. Thus, the effective deployment of methods to identify early infection of Xf is currently an important and innovative area of research. Epidemiological models have been used to predict pathogen spread and test surveillance strategies, but they rely on parameters estimated from sparse and imperfect data collected via ground surveys with limited budgets. In this sense, remote sensing (RS) has shown potential as a new form of large-scale surveillance, being able to identify pre-visual stages of disease development. However, RS information does not account for the connectivity of host landscape and the impact that the connectivity of susceptible host populations has on disease dynamics. This study demonstrates how combining epidemiological models, which capture the impact of connectivity and spatial disease dynamics, with RS, obtain better predictions of spatial disease distribution than both methods separately. The estimation method is based on an iterative stochastic optimisation algorithm to estimate the spatial distribution of Xf infection from a sample by explicitly simulating the individual distance-dependent spread processes between the pathogen and its host population. The present study adds some innovative aspects to this approach: the contribution of host connectivity on the pathogen spread throughout the landscape by circuit and graph theories and RS-based infection estimations using data provided by XF-ACTORS.

### **A preliminary investigation on the potential spread of *Xylella fastidiosa* in the UK using a multi-scale spread model**

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**Abstract:** *Xylella fastidiosa* is renowned to infect  $> 500$  plant species and be transmitted by ubiquitous insect species. The infection, once restricted to the Americas, is now quite rapidly spreading in new areas such as Southern Europe, most likely due to the introduction of infected plants from endemic areas.

Currently, the UK is believed to be free of the pathogen, but considering the extensive plant trade, and the potential effect of climate change on bacterium/vector ecology and distribution, it is highly possible that the bacterium will emerge in the UK, and, if undetected, is expected to cause major damage to crops, ornamental plants, and forest trees.

This work is a preliminary investigation on the potential spread of *Xylella* once introduced in the UK, using a multi-scale spatially explicit spread model. Predicting the spread and

estimating the efficacy of control measures is paramount to avoid potential outbreaks and have a better understanding of the epidemiology of the pathogen in such different environmental conditions (i.e. compared with geographical regions where *Xylella* was previously detected). Being pathogen-free, there are no epidemiological data regarding the UK, therefore we used all the available information to formulate qualitative and quantitative predictions, and theorise different scenarios. In particular, we model short-distance dispersal, stochastic long-distance dispersal, control zones (in agreement with the methodology employed in Apulia), and different levels of surveillance and detection efficacy.

### **Modelling xylem temperature in olive and almond trees to estimate *Xylella fastidiosa* infection in woody hosts**

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects PONTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** *Xylella fastidiosa* (Xf) can infect a wide range of woody crops that are grown extensively worldwide. The temperature patterns inside the woody plant parts which are the habitat of Xf cells may nevertheless be different to those of the standardly recorded air temperature, due to the wood thermal inertia, wind speed or incident radiation energy and its shading by the canopy. To investigate the relationship between the temperatures that can be reached in different parts of the xylematic system and those of the surrounding air, an experiment was conducted in Córdoba, southern Spain, from 2017 to 2019. In a high-density plot, a set of four mature olive trees (two with maximum exposure to sunlight on the southern border and two in the middle of the plot) were instrumented with a multiplexed set of specially-made thermocouples placed in different parts of the active xylem tissues. Measurements were made uninterruptedly from winter 2017 to spring 2019 at 10-min. intervals: inside small branches (1 cm diameter), inside the trunk at 2 and 4 cm depths, and in the soil underneath the trees at 20 cm depth. This set was replicated on the southern (sunlit) and northern side of each tree; *in situ* standard measurement of air temperature at 1.5 m height was also recorded. The experiment was replicated in a nearby almond tree plot. Solar radiation, air temperature and wind speed were measured concurrently in a nearby meteorological station. Our results indicate that winter standard air temperature minimums closely match those of all the above-ground xylematic systems, while maximums may differ substantially (> 20 °C), especially in the deeper xylem rings. For these parts and the root xylem, a model of heat transfer which includes the shading effect may be necessary. These results would allow a better parametrisation of epidemiological models used to estimate the development of Xf infection in woody hosts.

#### *Acknowledgement*

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## **Session: Insect vectors**

### **Vector parameters relevant to model the management of *Xylella fastidiosa pauca* ST53 invasion**

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**Abstract:** We present a model, based on both Life Table and Statistical Mechanics techniques, aimed at identifying a valid Integrated Pest Management strategy to mitigate



or stop the *Xylella fastidiosa pauca* (Xfp) ST53 OQDS invasion. The model represents closed and open systems as simple square lattices that mirror actual olive orchard structure, with different boundary conditions. Main orchard parameters involved in the model are: trees per ha, length, number of branches and twigs, olive susceptibility to vector infestation and Xfp ST53 propagation rate in xylem. Vector parameters belong to the bionomics of *Philaenus spumarius* (Hemiptera Aphrophoridae) the main Xfp ST53 vector and are: population size, prolificacy, post-embryonic death rate, time to eclosion, feeding time, active dispersion, vector infection probability. Control parameters focus on juvenile vector control, adult vector control, control action timing and frequency, treatment efficacy, vector survivorship. Vector population control is performed through specific egg and juvenile population management actions, diminishing the overall population size. Transmission management is performed by means of chemical actions (i.e. spray or injection of synthetic xylem-moving insecticides, selected by experimental mortality data) that kill the adults during the acquisition on the tree and minimise the following infection spread. The control of adults is challenging because *P. spumarius* acquires and performs infections and further transmissions almost without latency, depending on the status of the plant they encounter. The impact of orchard structure, number and frequency of different means of discouraging or impeding the transmission are also discussed. This study shows that different tailoring, timing and tuning of available control actions lead to different invasion control efficacies. Nevertheless, harmonising within an IPM strategy the control of both vector population and transmission can mitigate the infection, ending the pathogen invasion, eventually.

### **Relationship between vectors of *Xylella fastidiosa* and the almond leaf scorch disease in the demarcated area in the province of Alicante (Spain)**

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** *Xylella fastidiosa* was identified for the first time in mainland Spain in 2017, in the northeast of Alicante province, on almond trees affected by leaf scorch. Since then, the disease evolution and the potential vectors of the bacteria have been monitored periodically in the dDemarcated Area. This work shows the updated results on the surveillance and analysis campaign carried out from 2017, based on the sampling of almond trees and nymphs and adults of potential vectors, captured both on the vegetation cover and on the trees from several orchards. The vector species present in the area have been identified, and both the insects and their host plants have been analysed for *X. fastidiosa* detection.

Until now, *X. Fastidiosa* has been detected in two insect species, which some authors have previously shown to transmit the pathogen: *Philaenus spumarius* and *Neophilaenus campestris*. The relative presence of the two vectors in the samplings was similar: 42% for *P. spumarius* and 54% of *N. campestris*. However, the bacterial infection percentage in the analysed individuals was quite different: 10.49% in *P. spumarius* and 0.75% in *N. campestris*.

With global results, the relationship between these potential vectors and the distribution of the infection in the different zones of the Demarcated Area, are being analysed.

### **A taxonomic perspective of potential *Xylella* vectors in Europe**

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**Abstract:** The focus of research on *Xylella* vectors has understandably focused on *Philaenus* species in western Europe, and especially *Philaenus spumarius*. Other species of

*Philaenus* have also received some attention in Italy and in Spain and Portugal. This presentation will discuss the taxonomic distinction between the *Philaenus* species. It will also highlight the other potential xylem-feeding vectors in western Europe.

### **Characterisation of *Xylella fastidiosa* vectors in the Balearic Islands using the DNA barcoding approach**

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**Abstract:** *Xylella fastidiosa* is a pathogenic bacterium that infects the xylem vessels of a wide variety of plant species causing numerous diseases which, in most cases, produce serious damage both physiological and economical. In Spain, this emerging pathogen of American origin was first detected in 2016 in Majorca. Since then, repeated outbreaks have been found in Menorca, Ibiza and Alicante. Initial studies suggest that the Balearic Islands have a different scenario from what happens in other parts of the world with 21 affected plant species and three subspecies of this bacterium. There are many unresolved questions about the epidemiological factors that have determined the bacterium colonisation in the Islands. Molecular tools have been used successfully to answer questions about the epidemiology and ecology of vector-borne diseases. Our work focuses on identifying the vectors of *X. fastidiosa* in the Balearic Islands using the technique of DNA barcoding, which consists of the amplification of a 650-base-pair fragment of the mitochondrial genome. To date, we have sequenced more than 200 samples. The results of our study confirm the molecular identification of the *X. fastidiosa* vector species in the Balearic Islands (*Philaenus spumarius*, *Neophilaenus campestris*) and we present the phylogenetics of these insects.

### **Morphological and genomic assessment of divergence between closely related species of the genus *Philaenus* (Hemiptera, Aphrophoridae)**

Seabra SG\*, Neto C, Rodrigues ASB, Streito J-C, Genso G, Pierre E, Silva SE, Marabuto E, Figueiredo E, Mateus C, Wilson M, Rei F, Quartau JA, Paulo OS, Rebelo MT

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**Abstract:** *Philaenus spumarius* (Linnaeus, 1758) (Hemiptera, Auchenorrhyncha, Aphrophoridae), the meadow spittlebug, is a polyphagous xylem-feeding insect, quite widespread in the Holarctic region. It has lately been under attention since this species was found to be a vector of *Xylella fastidiosa* during a recent outbreak in Italy. Several other species of the genus *Philaenus* are found in southern Europe, most of them being differentiated by morphological, karyological and genetic characteristics. Two of the species, *P. spumarius* and *P. tessellatus* are considered sister species, differentiated at the male genitalia level but not at karyological, mitochondrial DNA and some nuclear gene levels. Knowledge about the amount of morphological variation in the male genitalia shown in both of these species is, however, still lacking. Here we aim at characterising such variation in populations from the southern Iberian Peninsula, where both species occur. New data on thousands of genome-wide markers (RAD sequencing) revealed previously unreported differentiation between *P. tessellatus* from Morocco (defined by male genitalia) and *P. spumarius* from the Iberian Peninsula, contrasting to an absence of mtDNA differentiation. It is expected that such data may provide genetic markers for more accessible genotyping of specimens to discriminate these two closely related species, with relevance in the management of *X. fastidiosa* vector populations.

### **Surveillance of *Xylella fastidiosa* vectors in the Balearic Islands (Spain)**

Tugores MA, Seguí G, Menéndez A, Barceló C, Delgado-Serra S, Paredes C, Lester K, Kenyon DM, Lalucat J, Gomila M, Miranda MA\*

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**Abstract:** *Xylella fastidiosa* is a xylem pathogenic gram-negative bacterium that is vector-transmitted. The bacteria cause several important diseases in crops. In October 2016, *X. fastidiosa* was detected in the Balearic Islands, including the subspecies *fastidiosa*, *multiplex* and *pauca*. In 2017, a project aimed to increase knowledge on the bioecology of potential vector species in the Balearic Islands was sponsored by EFSA. Annual biweekly surveillance of vectors has been conducted in olive, almond and vineyard orchards in Majorca, as well as isolated samplings in Minorca and Ibiza. Vectors were sampled in both the herbaceous cover and in the tree canopy. All captured vectors were analysed by quantitative PCR in order to detect the presence of *X. fastidiosa* in the foregut. We have confirmed the presence of three species of Aphrophoridae: *Philaenus spumarius*, *Neophilaenus campestris* and *N. lineatus* in Majorca, Minorca and Ibiza. Nymphs of these species are mainly found from March up to May. The main plant species that host nymphs are *Chrysanthemum* spp. and *Sonchus* spp. for *P. spumarius* and for *Neophilaenus* spp. only Gramineae. Adults were found in the cover vegetation from May to early June and from September to November for oviposition. Adults of *P. spumarius* and *N. campestris* were found in trees from May to July, but only *P. spumarius* has been found in abundance, which is why it is the only relevant vector for the secondary transmission of *X. fastidiosa*. In terms of risk of transmission due to vector abundance, olive crop showed the highest population of adult vectors, followed by vineyard and almond crops. The average of *X. Fastidiosa*-positive (vector positive/total vector analysed by crop) *P. spumarius* was 0.04, 0.04 and 0.05 in olive, vineyard and almond crops respectively, while for *N. campestris* we found 0.06, 0.03, and 0 respectively in the above-mentioned crops.

### Distribution and identification of potential vectors of *Xylella fastidiosa* in almonds, vineyards and citrus in mainland Spain

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** The introduction of the vector-borne bacterium *Xylella fastidiosa* on the European continent is causing important economic and social disturbances, especially in the olive groves of Southern Italy. The recent detection of the pathogen in several key crops in mainland Spain such as almond, apricot and plum in Alicante and in olive in Madrid has increased the need to assess the degree of threat to which Spanish agriculture is exposed. Therefore, a deep knowledge about the distribution and population trends of potential vectors (Hemiptera: Cicadomorpha) is required to assess the risk of pathogen spread. In addition to the recently published results in olive groves, almond groves (Madrid and Alicante), vineyards (La Rioja and Madrid) and citrus groves (Murcia) were sampled from 2017 to 2019 in order to identify the population trends of the potential vectors of *X. fastidiosa*. Nymphs of spittlebugs were sampled every 10 days starting from early March until spittles were no longer observed. The number of spittles per plant, number of nymphs per spittle, and spittle position on the ground vegetation was counted. Adults of Cicadomorpha were sampled both in the ground vegetation and the tree canopy by using the sweep net method from early April to November. The spittlebugs *Philaenus spumarius* and *Neophilaenus campestris* (Hemiptera: Aphrophoridae) were the main vector species associated with the three crops. Furthermore, we found the potential vector *Cicadella viridis* (Hemiptera: Cicadellidae) on the ground vegetation associated with the vineyards located in La Rioja (Northern Spain). Preliminary results indicated that the highest vector density was observed in almond groves of Alicante. We found a high density of nymphs of

*P. spumarius* and *N. campestris* from early March to late April reaching densities higher than 100 nymphs/m<sup>2</sup> in ground vegetation below the almond trees. However, the rest of the areas sampled presented a much lower density of vectors ranging from peaks of 1.3 nymphs/m<sup>2</sup> in almonds to 4.8 nymphs/m<sup>2</sup> in vineyards.

### **Effect of mechanical control on *Xylella fastidiosa* vector population in Mallorca (Balearic Islands, Spain)**

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**Abstract:** *Xylella fastidiosa* (Proteobacteria: Xanthomonadaceae) is a xylem pathogen bacterium transmitted by xylem-feeder insects that causes several important plant diseases such as Pierce's disease in grapes or leaf scorch in almond trees. The bacterium was detected in the Balearic Islands in October 2016, including three subspecies: *fastidiosa*, *multiplex* and *pauca*. In 2018, a national project financed by INIA started with the aim of exploring control strategies of the vectors. The major potential vectors described in the Balearics are *Philaenus spumarius* and *Neophilaenus* spp. In order to interfere with the life cycle of vectors, we tested the effect of mechanical control of the plant cover on the most vulnerable phases, such as nymphs and/or newly emerged adults. For this, we selected two organic and one conventional olive orchard, as well as two organic and one conventional vineyard in Mallorca. Owners of each selected plot conducted mechanical control according to their common procedures and their own machinery, which in general included cut and tillage of the plant cover during March–April. Nymph abundance per surface (30 sampling points/orchard x 0.25m<sup>2</sup>) was measured in each plot on a weekly basis before and after mechanical control.

The trial is still ongoing. Preliminary results indicated that average densities before mechanical control were 1.9 nymph/m<sup>2</sup> and 0.7 nymph/m<sup>2</sup> in the two organic vineyards, while 0.3 nymph/m<sup>2</sup> were found in the conventional vineyard. For olive crops, 6.7 nymph/m<sup>2</sup> were found in the conventional olive orchard, while 1.5 nymph/m<sup>2</sup> and 10.1 nymph/m<sup>2</sup> in the organic olive orchards.

### **Understanding the distribution and ecology of insect vectors of *Xylella* in the UK**

Stewart AJA\*, Wilson MR

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**Abstract:** The froghopper/spittlebug *Philaenus spumarius* is widely regarded as a vector of *Xylella fastidiosa*. Although not yet reported from the UK, if ever introduced, *Xylella* would have devastating consequences for the horticultural and forestry industry, and potentially also for native biodiversity. *P. spumarius* is very widely distributed within the UK, but there is a need to better understand its use of different host plant species and its potential for dispersal. I will describe the activities with the BRIGIT project that are designed to map the geographical distribution of *P. spumarius* and the other c. 20 species of xylem-feeding insect occurring in the UK that are potential vectors of *Xylella*.

### **Presence and diversity of Xf vectors, their vegetal hosts and functional diversity, under climatic conditions foreseen for the Iberian Peninsula due to the global warming effect**

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**Abstract:** *Xylella fastidiosa* Wells et al. (Xf), as other phytopathogenic bacteria, is not directly controllable so disease management still largely relies on eliminating alternative

hosts, suppressing xylem-feeding vector populations and using resistant cultivars. Its emergence in Europe with considerable negative impact on olive culture has emphasised the importance of extended monitoring of susceptible host plants and potential vectors, in regions with favourable conditions for bacterium spread. Under this assumption this study aimed to evaluate the presence and diversity of Xf vectors, their hosts and the potential role of functional diversity associated with the vector's natural enemies, under climatic conditions like those foreseen for the Iberian Peninsula due to the global warming effect. Invertebrates were vacuum sampled during the autumn of 2016, from olive canopies and weeds, in 117 sampling sites distributed by an olive production region with more than 16 thousand square kilometres, where 585 olive trees were sampled, after two successive years with summers and autumns with temperatures and drought above average. Auchenorrhyncha, predators and parasitoids were identified to the lowest taxonomic level possible. *Philaenus tessellatus* Melichar and *Neophilaenus campestris* (Fallén) were the identified potential Xf vectors. Abundance of vectors and natural enemies was compared between olive canopy and weeds through GLMs. Mean abundance was generally higher on weeds with statistically significant differences for all analysed taxa, except for Platygastroidea (Hymenoptera), Coccinellidae (Coleoptera) and Aranea. This study showed the resilience of potential Xf vectors under conditions of high temperature and dryness associated with global warming conditions, as well as the notorious importance of weeds as hosts to vectors, despite their scarcity and availability, and reveals the existence of natural suppression relationships between potential vectors and parasitoids, even in these climatic conditions.

### **Improvement of a real-time lamp protocol for the detection of *Xylella fastidiosa* in *Philaenus spumarius* and *Neophilaenus campestris***

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnte 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** The epidemic spread of *Xylella fastidiosa* (Xf) in southern Italy, with very important economic repercussions for the olive tree industry, makes it advisable to use methodologies for early monitoring of potential tree infection before symptom development in host plants, such as the use of spy insects. This approach is based on the use of molecular tests to detect the presence of Xf, among which the real-time LAMP. In this work, a commercial kit (Enbiotech, Italy) based on this technique, was assayed for detection of Xf in *Philaenus spumarius* and *Neophilaenus campestris* specimens in different demarcated areas in Europe. Spiked samples were tested using the entire insect and bulk insect heads artificially inoculated with serial dilutions (from 10<sup>6</sup> to 10<sup>0</sup> CFU) of a strain of *X. fastidiosa* subsp. *pauca* ST53, isolated from an olive tree in Apulia. In order to exclude the loss of sensitivity due to the presence of inhibitors in the reaction, spiked samples were also tested using the insect heads macerated in the extraction buffer provided by the kit. The lowest bacterial dilution was always detected. Approximately 525 individuals of *P. spumarius* were sampled in late summer in an infected olive grove in Lecce (Italy) and tested by this assay. The total incidence of infection ranged from 13% to 16%, using single entire insects or single heads, respectively. This incidence was confirmed with bulk heads to assess the diagnostic sensitivity of the real-time LAMP test; with a progressive increase in bacterial detection observed by analysing a higher number of heads. Finally, heads of approximately 280 *P. spumarius* and *N. campestris*, collected in an infected almond grove in Alicante (Spain), are being analysed to determine the infection prevalence using the commercial kit and the real-time-PCR of Harper et al. (2010, erratum 2013), to compare the sensitivity of both techniques.

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Harper

## Presence and seasonal abundance of Auchenorrhyncha in olive groves in Greece

Afentoulis D, Stamatakou G, Vetsos P, Theodorou D, Konidis G, Siapkara D, Tribonia S, Koufopoulou P, Thanou Z, Kalaitzaki A, Zarboutis I, [Tsagkarakis A](#)\*

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**Abstract:** Studies of the species composition, seasonal appearance and abundance of Auchenorrhyncha in olive crops is of paramount importance to reduce the potential of *Xylella fastidiosa* to invade new areas. As similar investigations had not previously been conducted in Greece, extensive surveys were undertaken in olive orchards located in some of the most important regions for olive production in central Greece. Surveys took place using malaise traps examined on a monthly basis. Results showed high levels of species richness in the olive orchards, and the Auchenorrhyncha diversity varied among the regions surveyed. Most of the species listed as potential vectors of *X. fastidiosa* in Europe were found in relatively low numbers. Furthermore, many insects of the Deltocephalinae subfamily were found, whose behaviour as vectors should be further studied. The dominant and most frequent species found in the three regions were tested and found not to be associated with transmission of the bacterium.

## 'Nemaguard' xylem sap is not an effective mouth wash for *Xylella fastidiosa*-infected vectors

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**Abstract:** 'Nemaguard' was selected by the United States Department of Agriculture and released in 1959 as a rootstock for almond and stone fruits due to its resistance to nematodes and enhanced scion vigour. 'Nemaguard' happens to be resistant to *Xylella fastidiosa*, the causal agent of almond leaf scorch disease. Previous research showed that prior to June-budding this rootstock can prevent infection of almond nursery stock by *X. fastidiosa*. Further, the rootstock also promotes an apparent complete pathogen elimination and remission of leaf scorching symptoms in infected susceptible scions. However, nothing is known about the potential chemical and physical mechanisms of resistance to *X. fastidiosa* infection. The goal of the current study was to determine whether insect vector feeding periods on 'Nemaguard' can reduce bacterial populations in vectors or its transmission efficiency to susceptible plants. After acquiring *X. fastidiosa* from infected grapevines, vector access periods of up to 14 days on 'Nemaguard' did not reduce pathogen population densities in vectors or transmission efficiency of *X. fastidiosa* to susceptible plants when compared with the control. Results suggest that natural chemical properties of 'Nemaguard' xylem sap are not involved in resistance to *X. fastidiosa* and that future research should focus on identification of potential physical traits that prevent bacterial multiplication or movement within the plant.

## *Ooetonus vulgatus* (Hymenoptera, Mymaridae), a potential biocontrol agent to reduce the populations of *Philaenus spumarius* (Hemiptera, Aphrophoridae) in Europe

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**Abstract:** As a vector of *Xylella fastidiosa* (Wells, 1987) in Europe, the meadow frog hopper, *Philaenus spumarius* Linnaeus, 1758 (Hemiptera: Aphrophoridae) is a species

of major concern. Therefore, tools and agents to control this ubiquitous insect that develops and feeds on hundreds of plant species are wanted.

So far, only a few parasitoids of *P. spumarius* have been recorded. Adults are attacked by *Verrallia aucta* (Fallén, 1817) (Diptera: Pipunculidae) with parasitism rates reaching 70% in England. A few oophagous species have been recorded in the US: *Ooctonus vulgatus* Haliday, 1833 (Hymenoptera: Mymaridae) and at least two unnamed species of *Centrodora* (Hymenoptera: Aphelinidae).

We conducted a field survey of *P. spumarius* eggs in Corsica and provide a first report of *Ooctonus vulgatus* as a potential biocontrol agent of this species in Europe. We summarised the main characters distinguishing *O. vulgatus* from other European species to allow species identification by non-specialists and generated COI DNA barcodes to accurately identify the species.

We assessed parasitism rates at several sampling sites, highlighting the top-down impact of *O. vulgatus* on *P. spumarius* populations. Finally, we extracted temporal and geographic occurrences of *O. vulgatus* from the literature. If *O. vulgatus* is species-specific, sampling dates suggest that this parasitoid is univoltine. Indeed, adults are sampled in the spring, when they emerge from overwintering eggs, and in the fall, when they arise from estivation and probably oviposit in freshly laid eggs.

Based on these occurrences, we calibrated an ecological niche model to assess the potential distribution of *O. vulgatus* in the Holarctic region and to identify the main climatic drivers of its geographical distribution.

As a perspective, we plan future field surveys to ascertain the host specificity of *O. vulgatus* and confirm the potential of this species for the biocontrol of *P. spumarius*.

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Fallén

Wells

### **Seasonal abundance, spatiotemporal distribution and vector management tactics of Auchenorrhyncha potential vectors of *Xylella fastidiosa* in Crete (Greece)**

Kalaitzaki A\*, Koufakis I, Vasakis E, Tsagkarakis A, Broufas G, Pappas M, Tzobanoglou D, Roditakis E

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**Abstract:** Studies on seasonal abundance, spatiotemporal distribution and management tactics of Auchenorrhyncha potential vectors of *Xylella fastidiosa* is of paramount importance to reduce the potential of the disease to invade new areas. A survey of Auchenorrhyncha, which could potentially act as vectors of *X. fastidiosa*, was carried out over a 24-month period in olive groves in Crete, a major olive growing region in Greece. Adult populations of Auchenorrhyncha species have been monitored monthly since June 2017 using malaise traps and sweeping net. Auchenorrhyncha were present throughout the sampling period and their populations showed two peaks in November and June in both years. The most abundant subfamilies were Deltocephalinae and Typhlocibinae (Hemiptera: Cicadellidae) whereas Aphrophoridae, specifically the species *Philaenus spumarius* and *Neophilaenus campestris* were very low in numbers. Notably, Auchenorrhyncha were more abundant in the ground vegetation than in the olive tree canopy and adjacent natural vegetation and their populations were higher in western Crete than in the eastern part of the island. Our results provide new insights into the species diversity, ecology and population dynamics of potential vectors of *X. fastidiosa* in the olive groves of southern Greece, as well as important results for developing rational and efficient strategies against the spread of *X. fastidiosa*, enhancing the readiness of the authorities to eradicate the pathogen if that is required. To this direction, 23 chemical insecticides from

14 different modes of action are currently screened for their efficacy against three Auchenorrhyncha species, using a leaf-dip laboratory bioassay protocol. At least seven insecticides tested at the maximum recommended label rate exhibited mortality levels of over 95% against the species tested, suggesting they could be used to control *X. fastidiosa* vectors in a pathogen eradication scheme.

### Contribution to the knowledge of *Xylella fastidiosa* potential vectors in the Balearic Islands (Spain)

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**Abstract:** *Xylella fastidiosa* was first detected in Spain in October 2016 in Majorca (Balearic Islands) (Olmo et al., 2017). Since then, a large number of Xf positives has been detected; being identified two subspecies in Mallorca (*multiplex* ST7 and 81, and *fastidiosa* ST1) Xf subsp. *multiplex* (ST81) in Minorca, and Xf subsp. *pauca* (ST80) in Ibiza. Host plants include many vegetal species (EFSA, 2018). It is naturally transmitted by xylem 'specialist' insects belonging to the suborder Auchenorrhyncha (Hemiptera) (EFSA PLH Panel, 2015). In Italy, *Philaenus spumarius* (Hemiptera: Aphrophoridae) has been described as the main vector of the CoDiRO strain (Xf subsp. *pauca*) in olive orchards (Cornara et al., 2017), even though recent studies have shown that *P. italosignus* and *Neophilaenus campestris* can transmit Xf to some plant species (Cavaliere et al., 2018). A previous study performed in different orchards from the Balearic Islands revealed that *P. spumarius* together with *N. campestris* were the most abundant Aphrophoridae species (Miranda et al. 2017), being the main candidates for Xf transmission. We present data from vector surveillance carried out during 2018 in order to increase prior knowledge about potential vector species in almond crops from Majorca, its population dynamics and the frequency of naturally infected insects (still in progress). Analysis of collections showed that *P. spumarius* and *N. campestris* were the most abundant Aphrophoridae species in sampled orchards. *P. spumarius* nymphs were observed from the beginning of the sampling period (March) until late April, and adults were captured from mid-April to mid-November. On the other hand, transmission tests with *P. spumarius* were performed with the aim of establishing the local population's capacity to transmit Xf subspecies under semi-field conditions (greenhouse). Results showed that Xf was efficiently transmitted to healthy plants by infected *P. spumarius* vine to vine and almond to almond.

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## What are the potential vectors of *Xylella* in France? Overview of the results of a trapping network 2017–2018

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** From 2017 to 2018, Anses-LSV has implemented a national network for sampling potential vector insects of *Xylella fastidiosa*. Insects were collected in cultures and their nearby environment by different partners. About 385 sites were monitored with 1,563 samplings (all origins and years combined) in 10 of the 13 regions in metropolitan France. The top three participating regions are Occitanie, Nouvelle Aquitaine and Provence-Alpes-Côte d'Azur.

Samples were taken in agricultural crops and in the immediate environment/hedges. Several partners also carried out occasional samplings in non-agricultural or other areas.

When the location of the sampling is known, the main crops visited are grapevine (38%), and orchards (20%). Other samples include other crop types (including olive groves) but also uncultivated sites (e.g. scrubland, forest edges, lawns). The main technique used is the sweeping net in most situations.

A total of 31,207 insects (larvae and adults) were collected, including a majority of insects belonging to potential vector families. They were identified by morphological means. After exclusion of larvae (that can often not be determined morphologically) and cicadellid subfamilies not xylem-feeders (12 of 15 subfamilies in Europe), more than 9,000 potential vectors were studied.

The most common potential vectors in France are *Philaenus spumarius*, *Cicadella viridis*, *Neophilaenus campestris* and *Cercopis vulnerata*. We confirm here that *P. spumarius* is the most common species in France amongst potential vector species. We tested four methods to collect insects in the fields. No method is specific to Xf vectors. The method which combines the most abundant harvest and specificity, is the sweeping net. The Barber trap is only suitable for leafhoppers living at the soil surface and yellow pan traps and sticky traps are not sufficiently specific. Based on the network, we also provide basic phenology for the most common species.

## Spittlebughunt: survey of *Xylella* vectors and their host-plant preferences in Scotland

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**Abstract:** *Xylella fastidiosa* is a bacterial pathogen responsible for various plant diseases such as Pierce's disease, phony peach disease, and citrus variegated chlorosis in the Americas and for olive quick decline syndrome in Europe. The bacterium is transmitted by xylem-feeding insects belonging to the Order Hemiptera, suborder Auchenorrhynch. While the vectors are well understood in the Americas, our knowledge is less comprehensive in Europe. The meadow spittlebug *Philaenus spumarius* was the first vector species identified in Italy (Saponari et al., 2014) with other Aphrophoridae such as *Neophilaenus campestris* following (Elbeaino et al., 2014). A EUPHRESCO project was initiated in order to obtain general background information on the abundance and diversity of vectors; ultimately to assess the potential impact and spread of *Xylella* if introduced. Within the UK a citizen science survey was carried out on potential vector species investigating geographical

distribution and plant hosts. Samples of 'cuckoo spit' containing the insect nymph along with the associated plant material were collected and sent in for analysis. This allowed identification of both spittlebug and the plant it was feeding upon. Currently over three hundred spittlebug nymphs and one hundred adults from across Scotland have been processed. Adults are identified morphologically and DNA is extracted non-destructively, meaning reference specimens can be kept intact. Identification of adults using dual morphological and molecular techniques allowed the creation of a reference library of sequences and development of qPCR for quick screening of nymphs which are more difficult to identify. Preliminary results indicate that *P. spumarius* is the most abundant spittlebug species present in Scotland and is polyphagous, feeding on a wide range of plants including trees.

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Elbeaino

### **Seasonal occurrence of *Philaenus spumarius* and *Neophilaenus campestris* in olive orchards of Greece**

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**Abstract:** The introduction of the xylem-feeding bacterium *Xylella fastidiosa* in Europe has elicited investigations about the ecology of insect vectors that are potentially associated with the spread of the disease. In 2018 and 2019 samplings were carried out in olive orchards of central (Corinthia) western (Achaia) and southern (Chania, Crete) Greece to investigate: i) the seasonal occurrence of potential vectors of *X. fastidiosa* in Greece, ii) to identify host plants associated with the nymph's development. Samplings for nymphs are performed by collecting plants with spittle within a quadrat frame. Adults are collected using a sweep net and samples are taken both from the olive tree's canopy and ground vegetation.

In the areas of central and western Greece, *P. spumarius* and *N. campestris* nymphs were found on plants with spittle from mid-March until the end of April in 2018. In Chania Crete, only nymphs of *P. spumarius* were observed and they were present from late March until late April. Adults of *P. spumarius* and *N. campestris* were present in the areas of central and western Greece during April and May. However, during the summer months (June–September) they were absent from those olive orchards. They reappeared in early October and were captured until the end of December in those areas. In the sampling orchard of central Greece a few spittlebug adults were also captured during the winter months (January and February 2019). In the area of southern Greece, adults of *P. spumarius* were observed in April and from late October until mid-December. Regarding host plants, *P. spumarius* nymphs were observed mostly on plants of the Asteraceae and Fabaceae families while *N. campestris* nymphs mostly on plants of Poaceae family. Samplings for 2019 are ongoing.

### **Investigating the role of the meadow spittlebug (*Philaenus spumarius*) and its major host plant (*Cistus monspeliensis*) in the spread of *Xylella fastidiosa* in Corsica**

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**Abstract:** Using *Philaenus spumarius* (Hemiptera: Aphrophoridae) as a sentinel insect, we recently demonstrated that *Xylella fastidiosa* (Xf) was widely distributed throughout Corsica. During this survey, *P. spumarius* appeared to be the most abundant vector and field observations revealed that it mostly developed and fed on *Cistus monspeliensis*. We designed a large-scale survey to investigate the role of *P. spumarius* and *C. monspeliensis* in the dynamics of Xf.

Survey network: We monitored insect vector populations and symptoms on the vegetation three times a year on a network of 64 plots distributed in natural habitats across Corsica. The experimental plots were distributed along a density gradient of *C. monspeliensis* that has been suggested to be a potential and mostly asymptomatic reservoir. We also accounted for a possible effect of altitude and temperature on the presence of Xf, vector and plant community composition by setting up an altitudinal gradient from 0 to 600 metres as well as a vegetation gradient ranging from shrubland to forest.

Method: The intensity of Xf symptoms was estimated visually on four common plant species of the 'Corsican maquis: *Olea europaea*, *Quercus ilex*, *Myrtus communis* and *Arbutus unedo*. The population size of *P. spumarius* was estimated on each plot, through visual counting of foams or sweep netting of adults. Additionally, 30 specimens were sampled in the plot vicinity to: (i) assess Xf prevalence, (ii) characterise the Xf strain insects may carry, and (iii) identify which plants the insects fed on.

Results: Preliminary results suggest that: (i) *P. spumarius* abundance was positively correlated with *C. monspeliensis* density and turgidity, (ii) *O. europaea* and *A. unedo* expressed fewer symptoms than *M. communis* and *Q. ilex*, (iii) symptoms decreased during 2018, probably due to unusually high precipitations during spring and winter.

### **Assessing the local dispersal of *Aphrophora salicina*, a hypothetical vector of *Xylella fastidiosa* in Belgium**

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**Abstract:** Our working hypothesis is that *Xylella fastidiosa* might become established in Belgium, and if so, in a phytopathosystem which comprises *Salix* spp. and the aphrophorid hemipteran, *Aphrophora salicina*. So far, we have explored this possibility by inventories, detection tests on plants and insects, analyses of the acquisition of the bacterium by the insects and studies of life history traits of these latter.

Here we describe a new part of this study, aimed at developing a deeper understanding of the potential local spread of the bacterium via the natural movements of its hypothetical vectors.

In a first step, the adult insect population size was estimated. *Salix* twigs were sampled, on which egg loads were estimated by counting egg masses. Survival from the egg to the adult stage was then measured by following cohorts emerging from egg masses transferred to potted willow cuttings.

In a second time, flight capacity was measured in flight mills, repeatedly flying the same insects throughout different phases of their life cycle (emergent, mated, after oviposition).

Spatially explicit spread models based on real landscapes were then run using the parameter values gathered in the experiments described above.

### **Evaluation of the population of the meadow spittlebug *Philaenus spumarius* in Tuscan olive groves**

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**Abstract:** We investigated the factors shaping the abundance of *Philaenus spumarius* within four olive orchards in central and coastal Tuscany. For each of the selected locations a previous survey was performed to study the landscape composition surrounding the olive orchard at a small spatial scale (250 m). Observations of the juvenile instars were conducted following EFSA supporting publication 2019.EN1628. Adult insect sampling was performed by both entomological sweep net and by unbaited yellow sticky traps. Differences in *Philaenus* populations and their development in relation to site features and orchard management is discussed.

## Electrophysiological responses of insect vectors of *Xylella fastidiosa* to plant volatiles

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**Abstract:** The spittlebugs *Philaenus spumarius* and *Neophilaenus campestris* (Hemiptera: Aphrophoridae) are xylem-feeder insects that have been identified as vectors of *Xylella fastidiosa*. Plants emit a variety of volatile organic compounds (VOCs) that play multiple roles in plant-insect interactions; these semiochemicals are important cues for insects to locate an appropriate host plant, or mating and oviposition sites. Since spittlebugs have never been considered agricultural pests in Europe before the introduction of *X. fastidiosa*, their biology and ecology have been scarcely studied. So far, no research has been conducted to examine the important ecological chemistry behind these host plant-insect systems. In olive orchards in Greece, *P. spumarius* nymphs have been observed mostly on plants of the Asteraceae and Fabaceae families while *N. campestris* nymphs mostly on plants of the Poaceae family. In the summer months, adults of both species move from grass cover to woody plants and move back to grass cover in autumn. Using the dynamic headspace technique, we collected VOCs from *Olea europaea*, the major host plant for *X. fastidiosa*, and from various plant species that are used as cover crops or exist as natural vegetation in olive orchards, like *Cistus creticus*, *Medicago sativa*, *Cynodon dactylon*, *Festuca arundinacea* and *Sonchus oleraceus*. Additionally, we collected VOCs from *Pinus halepensis* that is common in the edges of olive plantations and from *Polygala myrtifolia* which is a highly susceptible plant to *X. fastidiosa*. Electrophysiological bioassays of both *P. spumarius* and *N. campestris* adults to the collected VOCs are scheduled using Gas-Chromatography coupled with Electro-Antennographical Detection (GC-EAD). Additionally, specific electrophysiological responses (EAG) of the insect's antenna to single chemicals identified in the volatile blends of the aforementioned plants are ongoing. Subsequently, the compounds that elicit a reaction in GC-EAD and EAG assays will be tested in behavioural experiments. The identification of semiochemicals that manipulate the spittlebug behaviour could contribute to the development of efficient monitoring tools for *X. fastidiosa* vectors.

## A survey of potential insect vectors of the bacterium *Xylella fastidiosa* in five regions of Tunisia

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**Abstract:** Xylem-sap-feeders hemipteran was a secondary threat for plant health, but today they are key pests for Mediterranean plant protection being vectors of xylem-inhabiting bacteria. The meadow spittlebug *Philaenus spumarius* L. (1758) (Hemiptera: Aphrophoridae) is the primary vector of *Xylella fastidiosa pauca* ST53, the causal agent of the olive quick decline in Apulia, while *Neophilaenus campestris* and *P. italosignus* are of secondary importance. We report findings of Auchenorrhyncha in Tunisia seeking for

possible *X. fastidiosa* vectors in southern Mediterranean shores. There were 1,757 Auchenorrhyncha results in 2017 and 2019 from collections in forests, cultivated or abandoned orchards or Mediterranean scrub of Djebel Boukornine, Djebel Chitana, Djebel Khroufa, and Djebel Abderahman. *Quercus suber* and *Q. faginea*, *Pinus halepensis*, *Pistacia lentiscus* and *Myrtus communis* dominate the plant associations. Collections by sweeping nets and mouth aspirators from cultivated and uncultivated orchards in the sandy, weedy environments near olive, peach, citrus, apricot, and ornamental trees were initiated in the regions of Bizerte, Béja, Tunis, Nabeul, Zaghouan, and in the park of Dar Chichou and Lac Ichkeul. Most of the collected species, 35%, belong to Cicadellidae. *Exitianus capicola* (Deltocephalid) was the most abundant species in olive, peach and apricot orchards. *Balclutha* spp. and *Cicadulina bipunctata* were abundant in citrus and olive orchards. The planthoppers (Fulgoroidea) were prevalent in forests with *Toya propinqua* and *Sogatella kolophon* the most abundant Fulgoromorpha species. The spittlebugs *P. spumarius* and *N. campestris* live in Djebel Chitana of Bizerte, in the park of Dar Chichou and Lac Ichkeul, and near the olive and citrus orchards of Zaghouan, Korbous and Kairouan. Thereby, in case of unwanted *Xylella* introduction, we expect the bacterium to invade the country given the presence of vectors, susceptible plants and favourable climate.

### Taxonomy and re-description of species within the genus *Philaenus*

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**Abstract:** *Philaenus spumarius* (Hemiptera: Aphrophoridae) is the efficient vector of *Xylella fastidiosa* in Italy and is found mixed with *P. tessellatus* and *P. maghresinus* in Tunisia.

This approach studied *Philaenus* collected by sweep net in Italy (Puglia) and in Tunisia to differentiate the three Tunisian species. Tunisian material originates from Ben Arous, Nabeul, Bizerte, Béja, Jendouba, Kairouan in *Pinus* and *Quercus* forests and also on *Pistacia* or *Asphodelus* understory, in olive, peach, citrus orchards and grapevines, and on natural herbs or weeds.

Apart from the interest in describing Tunisian fauna of Hemiptera, we need a clear taxonomic contribution based on the study of male genitalia for the identification of the 10 *Philaenus* species. The need arises because one of the species (*spumarius*) is a highly efficient vector of *Xylella fastidiosa*: a quarantine plant pathogenic bacterium threatening plant health in the Mediterranean basin. The correct specific identification will allow quantitative sampling and consequent effective control measures and strategy building.

The only comparative paper considers eight species and is useful for skilled researchers but less accessible for phytosanitary services or other students starting comparative *Philaenus* identification.

Here we focus and fully illustrate the morphological differences in male genitalia, targeting the shapes of: 1) aedeagus base (phallobase) from side and posterior view; 2) aedeagus shaft from side and posterior view; 3) number, shape, size, and points of insertion of aedeagal processes (projections); 4) endophallus window shape; and 5) anal tube in dorsal view of *P. spumarius*, *P. tessellatus*, and *P. maghresinus*.

Given the opportunity to discriminate the three taxa we found that *P. tessellatus* occurs in the northeast and west of Tunisia and is the most abundant species in the prospected regions. Furthermore, *P. spumarius* and *P. maghresinus* seem to occur associated in a limited area only in one locality in the north-east of Tunisia.

### Potential insect vectors of *Xylella fastidiosa* in Montenegro

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**Abstract:** As olive is the most widespread fruit tree in Montenegro, eventual detection of the bacterium *Xylella fastidiosa* would cause great economic losses, besides implying the implementation of a permanent monitoring campaign. This is especially due to the importance of early detection of the bacteria as well as its vector(s) for the timely implementation of control measures to prevent it spreading. Given the significance of the vectors in the transmission of *X. fastidiosa* and the limited information about their presence in Montenegro, a study was undertaken to determine the species of Auchenorrhyncha (Cicadomorpha) present in olive groves on the Montenegrin coast. During 2018, insects were collected using sweep net sampling at three locations in the area of Valdanos and two locations in the area of Radanovići (Sampling was carried out on weeds, twice in the area of Valdanos (late May and late October) and once in the area of Radanovići (early September)). The collected insects were preserved in 96% alcohol until determination. Insect identification was performed on the basis of the morphological characteristics of the species. Sixteen Auchenorrhyncha species were identified of which 12 belong to the Cicadomorpha, three to the Aproclyptidae and nine to the Cicadellidae families. In all three locations in the area of Valdanos the most prevalent was *Philaenus spumarius* L. (main vector of *X. fastidiosa* in Europe) while *Lepyronia coleoptrata* (L.) was mainly collected in the area of Radanovići (.

#### Acknowledgement

This work was supported by the Ministry of Science of Montenegro and CNR as part of the bilateral project Italy–Montenegro 'Prevention of the introduction and spread of the quarantine bacterium *Xylella fastidiosa* through plant propagation material' and Directorate for Food Safety, Veterinary and Phytosanitary Affairs.

### Phenology and host-plant selection of *Philaenus spumarius* in vineyards of north-western Italy

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**Abstract:** In order to contribute to the risk assessment of *Xylella fastidiosa* in vineyards, surveys of *Philaenus spumarius* were carried out in north-western Italy. A vineyard in Asti was sampled in 2016 and 2017. It was selected because of the ecological complexity of the site including grapevine rows, and herbaceous cover within and around the rows and broadleaved trees/shrubs surrounding the vineyard, as alternative hosts for the spittlebug adults. Nymphs and adults were sampled every 10 and 15 days, respectively, from the beginning of March until the beginning of December in both years. Nymphs were counted and their host plant identified, in herbaceous cover of vineyard headlands and inter-rows. Adults were sampled from three vegetation compartments of the vineyard: i) grapevine plants; ii) herbaceous vegetation (headlands and inter-rows); and iii) shrubs, trees and other spontaneous woody plants. Herbaceous cover was visually inspected for foam and nymphs, and each nymph was assigned to a life stage. Sampling of nymphs was done in randomly selected areas of 0.25 m<sup>2</sup>, delimited by a rectangular frame. Adults were collected by sweeping net, counted, sexed, identified and immediately released. All samplings were conservative. Population dynamics and phenology, with respect to chronological and physiological time, were described for both nymphs and adults. Host plant selection of nymphs and vegetation compartments of adults are reported all over the two years. Five more vineyards in the Piedmont Region, besides the one in Asti, were inspected in 2018 using the same methodology, although surveys were carried out only three times in the season. The first survey was conducted in April, at the time of nymph population peak. The second and third surveys were aimed at sampling adults in June and September. Population abundance and host plant selection by nymphs, as well as population abundance and vegetation compartments of adults are reported.

### Assessment of the genetic diversity in populations of *Philaenus spumarius* collected from different areas

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**Abstract:** The spittlebug *Philaenus spumarius* is a widespread species in Europe, recently attentioned because of its relevant role in spreading the plant pathogenic bacterium *Xylella fastidiosa* (Xf), a new emerging pathogen threatening the landscape and several crops in southern European countries. Being the main species associated with the epidemic spread of this bacterium currently decimating olive trees in the Apulia region (southern Italy), molecular investigations have been carried out to characterise the populations occurring in the so-called *Xylella*-demarcated areas of Apulia versus those present in other regions currently free from *X. fastidiosa*. Analysis also included specimens received from Montenegro and Tunisia.

Three DNA markers were used, the mitochondrial genes COI and cytochrome b and the nuclear gene EF1- $\alpha$ , to amplify and sequence DNA fragments from a total of 60 specimens. Mitochondrial sequence analysis showed that all Italian specimens belong to the south-west clade (which includes the Mediterranean basin and western Europe) and, in particular, the majority of the Apulian specimens and those collected in Montenegro belong to the eastern Mediterranean subgroup, whereas the specimens collected in other regions of Italy belong to the western Mediterranean subgroup. Conversely, the Tunisian specimens fell in the eastern group (Anatolia/Caucasus). Similar genetic relationships were retrieved from the analysis of the EF1- $\alpha$  gene.

The data herein obtained, while confirming that the population of *P. spumarius* responsible for the epidemic spread of Xf in Apulia belong to a single phylogenetic group, integrate the large dataset of biological and ecological information collected from the studies intensified on this species in the last few years.

#### Acknowledgement

The authors would like to thank the project 'Capacity Building and Raising Awareness in Europe and in Third Countries to Cope with *Xylella fastidiosa* - CURE-XF' the European Union's H2020-MSCA-RISE-2016 under grant agreement N. 734353 for its partial support of this work.

### Predominance and natural infectivity of potential vectors of *Xylella fastidiosa* in olives in south-eastern Brazil

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnte 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** As xylem-feeders, several species of sharpshooter (Hemiptera: Cicadellidae: Cicadellinae) and spittlebug (Hemiptera: Cercopoidea) are potential vectors of the xylem-limited bacterium, *Xylella fastidiosa*, which is associated with olive quick decline syndrome (OQDS) symptoms in south-eastern Brazil. Here we investigated the epidemiological importance of these species based on faunal indices and natural infectivity with *X. fastidiosa* in olive orchards of the Mantiqueira Mountain Range (MMR) region, in São Paulo (SP) and Minas Gerais (MG) states. The insects were sampled with yellow sticky cards hung at two heights (0.8 and 1.6 m) on the outer branches of nine olive trees per orchard; the cards were replaced fortnightly from June 2015 to August 2017. Based on faunal analysis, those species classified as dominant, very abundant and very frequent were considered predominant. Samples (n=15) of three individuals of each predominant species were evaluated for the presence of *X. fastidiosa* by qPCR and conventional PCR. Out of 48 species (5,055 individuals) of sharpshooters and spittlebugs trapped in an orchard in São Bento do Sapucaí (SP), six are predominant (*Clastoptera* sp.1, *Amblyscartidia pardaliota*, *Macugonalia cavifrons*, *Paratubana luteomaculata*, *Scopogonalia paula* and *Subrasaca bimaculata*), whereas in an orchard in Maria da Fé (MG), 7 of 45 species trapped (2,026 individuals) are predominant: *Clastoptera* sp.1, *Bucephalagonia xanthophis*, *Erythrogonia*

*dorsalis*, *Erythrogonia phoenicia*, *M. cavifrons*, *S. paula* and *Oncometopia facialis*. *X. fastidiosa* was detected in samples of *Clasoptera* sp.1 (53%), *M. cavifrons* (20%), *P. luteomaculata* (50%), *S. paula* (20%) and *S. bimaculata* (33%) collected in São Bento do Sapucaí, but in none of the four predominant species collected in Maria da Fé. Combined results of predominance and natural infectivity show that the spittlebug *Clasoptera* sp.1 and the sharpshooters *M. cavifrons*, *P. luteomaculata*, *S. paula* and *S. bimaculata* may be epidemiologically relevant for disease spread in the MMR region, if confirmed as vectors of *X. fastidiosa* in olives.

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## Abundance of spittlebug nymphs (Hemiptera: Aphrophoridae) in Trás-os-Montes region, Portugal

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**Abstract:** Spittlebugs (Hemiptera: Aphrophoridae) are considered the main European vector of *Xylella fastidiosa*, a gram-negative bacterium responsible for serious diseases in important agronomic crops. Nymphs of spittlebugs develop in vegetation cover where they produce a spittle mass that provides protection from natural enemies and solar radiation. This spittle mass is visible to the unaided eye which facilitates its monitoring, the understanding of nymph's dynamics and the implementation of control strategies against *X. fastidiosa*. In this context, the goals of this work were monitoring the abundance of spittlebug nymphs and identifying the host plants in the natural ground cover in the Trás-os-Montes region, Portugal. For that, the presence, number of spittles per plant, number of nymphs per spittle, and spittle position low, medium or high were recorded over a sample unit of 100 × 25 cm in one olive grove (spring 2017 and 2018), in one almond orchard and one vine (spring 2018) from Trás-os-Montes. Thirty sample units randomly distributed over a transect covering 1 ha were selected per sampling date and sampling site. The peak of spittlebug nymphs occurred in the middle of April and first weeks of May in 2017 and 2018, respectively, in all sampling sites. Nine spittle masses and a mean of  $1.93 \pm 0.35$  plants with spittle per m<sup>2</sup> were recorded respectively in 2017 and 2018. In 2017, *Crepis vesicaria* L. and *Bromus diandrus* Roth were the most attacked species. In 2018, the presence of nymphs of the genus *Neophilaenus* was more abundant in the vine, presenting a mean of  $0.60 \pm 0.19$  nymphs per m<sup>2</sup>, while *Philaenus* nymphs showed a higher abundance in the olive grove, presenting a mean of  $0.17 \pm 0.08$  nymphs per m<sup>2</sup>. Nymphs of the genus *Neophilaenus* were identified with high frequency in the species *Avena barbata* subsp. *lusitanica* (Tab. Morais) Romero Zarco and *Cynodon dactylon* (L.) Pers and the *Philaenus* nymphs were identified more frequently in the species *Erodium cicutarium* (L.) L'Her. and in the genus *Trifolium* spp. It was verified that the number of nymphs registered in the Trás-os-Montes region was much lower than the numbers reported in other European regions, being necessary more years of study to understand the dynamics of spittlebug nymphs.

## Mobility of sharpshooter vectors of *Xylella fastidiosa* in areas adjacent to citrus groves in São Paulo State, Brazil

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**Abstract:** Mobility is an important attribute of a vector, allowing faster and farther spread of plant pathogens. *Xylella fastidiosa* is transmitted by a wide range of sharpshooters and



spittlebugs with different host plant and habitat associations, with implications for behavioural characteristics, e.g. mobility. In this study, we evaluated trap catches of sharpshooter vectors at 2.5 m and 7.5 m above ground as a measure of mobility, in areas surrounding citrus groves in two localities (Botucatu and Casa Branca) of São Paulo State, Brazil. Three yellow sticky cards of 10 x 30 cm were used per trapping height on several bamboo stakes positioned in areas surrounding citrus groves in the municipalities of Botucatu and Casa Branca of São Paulo State, Brazil. The sticky cards were replaced fortnightly from November 2017 to December 2018. Among > 10 species of sharpshooter and spittlebug trapped, *Acrogonia citrina* and *Oncometopia facialis* were the most frequent at 7.5 m, with total catches of 1,681 and 123 individuals, respectively. The proportions of individuals trapped at 7.5 m were higher for *A. citrina* (18–48%) than *O. facialis* (12–21%), with the highest values recorded in Botucatu. Insects were trapped at 7.5 m throughout the year, but more frequently in summer and/or fall. The results suggest that sharpshooters are strong fliers and may engage in wind-assisted flights, possibly carrying *X. fastidiosa* over long distances.

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### Surveys for vectors and candidate vectors of *Xylella fastidiosa* in olive orchards in Apulia

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects PONTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).

**Abstract:** For three consecutive years, surveys were conducted in 24 olive orchards located in the demarcated *Xylella*-infected area of Apulia (southern Italy), with the aim of identifying xylem-feeder species serving as vector of *Xylella fastidiosa* in olives. Surveys were carried out periodically from spring to late autumn and insects were collected by sweeping net using constant sampling units from olive canopies, ground vegetation and border plants when present. All insects were subjected to qPCR for the detection of *X. fastidiosa* (individually or in groups of 5), as well as to the morphological identification. Besides the two spittlebug vectors (*Philaenus spumarius* and *Neophilaenus campestris*), more than 3,000 specimens belonging to 37 different species (27 Cicadomorpha and 10 Fulgoromorpha) of 34 genera and 10 families were collected. The detection of the bacterium in spittlebugs, xylem-feeders and on some of the most abundant phloem-limited species, showed that: (i) for the two known vectors occurring in the area, a higher incidence of positive specimens was detected for *P. spumarius* (up to 50%) with the occurrence of *Xylella*-positive insects for a prolonged period, compared with *N. campestris* (10% of positive specimens, detected to a limited extent in late spring), and (ii) for both species the incidence of positive specimens was consistently higher when they were collected from olive canopies compared with ground vegetation or border plants; (iii) amongst the xylem-feeders, none of the specimens collected and tested for *Cicada orni*, *Cercopis sanguinolenta* and *Lepyronia coleoptrata* were found positive; (iv) conversely, few positive specimens were recovered for the following leafhoppers and planthoppers: *Thamnotettix zelleri* (8.33% in olives and 3.7% in weeds); *Latilica tunetana* (1.77% in olives, 2.2% in weeds) and for *Euscellis lineolatus* (none in olives, 0.06% in weeds). Regarding the possible role as vector of *X. fastidiosa* for these planthopper/leafhopper species, transmission tests have already excluded the capability of the *L. tunetana* to retain and transmit the bacterium, whereas experimental tests are ongoing for *T. zelleri*.

#### Acknowledgement

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 635646 'Pest Organisms Threatening Europe PONTE'.

## Tacking *Phylaenus spumarius* through geo-statistical analysis of field observations and identification of wild fennel as a key host herbaceous species during the insect early growth development

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**Abstract:** *Phylaenus spumarius* L., the meadow froghopper, is actually assessed as the main carrier of *Xylella fastidiosa* in the Mediterranean basin. In a previous study, aimed to improve the understanding of the insect's nutritional behaviour, especially at its critical nymph stage, by monitoring its presence on different herbaceous target species, we used typical feeding foams as key indicators (Latini et al. 2019). The study area was in the Lazio region (central Italy), dedicated to olive growing and still unaffected by the *Xylella* plague. In the last five years, during the nymph development period, field data have been acquired over the test areas and then analysed by coupling statistics, geographical information system (GIS) and georeferenced field sampling. Results highlight that *P. spumarius* exhibits some preferences for specific herbaceous plants, especially at its early development stages, detectable by a very tenuous spittle; thus it is possible to speculate that a defined mechanism leading to the plant choice exists, driving the females at the time of oviposition.

Applied implications arising from these findings for *P. spumarius* population management are discussed. Indeed, our attention has shifted the focus from the three well-known targets, i.e. the olive tree plantation, *X. fastidiosa* and *P. spumarius*, to a restricted identified group of herbaceous plants that look particularly attractive for the insect carrier at its early growth stage. As confirmed during further several field observations, under the different conditions of biodiversity present in various locations in central Italy, *Foeniculum vulgare* behaves as a key plant for the early larval stages of *P. spumarius*. Possible implications due to the invasive nature of wild fennel, as well as its recognition as 'emerging' alien species, are also argued.

*Bibliography*

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## Session: Risk and impact assessment

### Pest categorisation of non-EU Cicadomorpha vectors of *Xylella* spp.

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**Abstract:** Following a request from the European Commission, EFSA was asked to provide a pest categorisation of 'non-EU Cicadellidae vectors of Pierce's disease'. Currently only three species (*Carneocephala fulgida* – current name *Xyfon fulgida*, *Draeculacephala minerva* and *Graphocephala atropunctata*) are listed in Directive 2000/29 EC as examples of such vectors and are regulated as harmful organisms whose introduction into, and spread within, all Member States shall be banned. Because Pierce's disease is one of those caused by *Xylella fastidiosa* and this xylem-limited bacterium is transmitted to plants by insects, in principle all phytophagous insects with hemipteroid piercing-sucking mouthparts that accidentally probe xylem vessels (i.e. the order Hemiptera) with up to 100,000 species could potentially be considered for categorisation. However, the terms of reference were extended into the suborder Cicadomorpha (approximately 30,000 species) as up to now only xylem-sap-feeding families within this suborder (including Cicadellidae) contain vectors of this plant pathogenic bacterium. As vectors of *Xylella* spp. are not specific, the categorisation of the non-EU insects which are proven vectors elsewhere is of paramount importance because the potential for introduction into the EU has dramatically increased due to globalisation. The starting point of data collection was the EFSA *Xylella*

spp. host plant database 2018. Additional, more recent, sources were also explored. Published reports of successful vector transmission were taken as selection criterion. Around 50 species of this group, mostly Cicadellidae but also some Aphrophoridae and Membracidae, were categorised; their taxonomic status, geographic distribution, biology, potential impact in the EU and risk reduction options were evaluated in a systematic manner to determine whether they fulfil the criteria required to be considered for regulation as Union quarantine pests.

### **Pioneering information for prevention and containment of *Xylella fastidiosa*, a case study for the epidemic in Apulia (southern Italy)**

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**Abstract:** The difficulties in the implementation of containment measures over five years of a severe epidemic of *Xylella fastidiosa* in Salento (Apulia, southern Italy), as a consequence of uncontrolled free dissemination of fake news, demonstrated that timely and effective information and communication on the same ground utilised by the misinformation, social media, are strategic not only in contrasting the bacterium/vectors on the outbreaks but especially for prevention. From 2016 Infoxylella®, a spontaneous initiative of volunteers (the editorial staff include scientists, olive growers and agronomists), considering the complexity of scientific arguments, the continued rapid evolution of legislation and the confusion created by misinformation, try to inform public opinion and media with simple language, filtering the essential information only from official verified sources and profiting from a continuous collaboration with the coordination team of the H2020 projects POnTE and XF-ACTORS. Thirty-four municipalities, producer's organisations, associations, companies, etc. became formal supporters of Infoxylella, now in the process of becoming a non-profit organisation. Besides the growing popularity and success on the web and social media (i.e. the Facebook page called Infoxylella.it reached 10,000 followers in April 2019), Infoxylella became a reliable source of information for journalists (mentioned as a source in over 60 articles in national journals), other media and in particular is active in the territory participating in informative seminars organised by the municipalities (over 30 in 2018). In order to involve the local administrations in prevention and public information as collective contrast to the epidemic, Infoxylella prepared an informative booklet already adopted, for printing or publishing in electronic format, by 25 local entities. Despite its young age and the specificity on *Xylella* in Apulia, Infoxylella.it could be considered as a case study providing useful practical experience and ideas to improve elsewhere public awareness on phytosanitary issues and direct involvement of population in the contrast of other epidemics.

### **EFSA data collection on *X. fastidiosa*: risk assessment uncertainties, knowledge gaps and research**

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**Abstract:** Following the report of the first outbreak of *Xylella fastidiosa* outdoors in Europe in 2013, EFSA rapidly reacted to the emergency by delivering urgent advice (on host plants, entry and spread pathways and risk reduction options) and then a comprehensive pest risk assessment, which identified several risk assessment uncertainties. This led to the organisation, following a request from the European Commission DG RTD, of a workshop on knowledge gaps and research priorities and a series of cooperative research conferences to provide a common platform for researchers, risk managers and stakeholders to present and discuss research results and priorities for the control of *X. fastidiosa* in Europe. In addition, EFSA has launched the outsourcing of small pilot research projects to improve the understanding of the epidemiology of this bacterium. The first pilot project was conducted in 2013–2015 to study the host range of *X. fastidiosa* subsp. *pauca* ST53 in Apulia. The second project was dedicated to the collection of data on the biology and control

of *Xylella* vectors and potential vectors in 2016–2018. This included three years of data collection in fields in the Apulia, Liguria and Piedmont regions and in mesocosms and microcosms located in two research centres in Bari and Turin. In 2017 another EFSA-funded project started on the collection of data and information on the biology of vectors and potential vectors of *X. fastidiosa* in the Balearic Islands (Spain), with a focus on field surveys and vector transmission experiments. Data from vector transmission experiments are also systematically reported in the EFSA *Xylella* host plant database. With these activities, since 2013, EFSA has supported the collection and sharing of research data on *X. fastidiosa*.

### **Possible entry pathways and host plants of the bacterium *Xylella fastidiosa* in Bulgaria**

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**Abstract:** Possible pathways for entrance of *Xylella fastidiosa* into the territory of Bulgaria are mainly through the trade of infected plants for planting or through infected ornamental plants.

Potential vectors of this bacterium in the country are 27 insect species, from six families – Cicadellidae, Aphrophoridae, Cercopidae, Cicadidae, Tibicinidae and Membracidae; possible vectors are *Cicadella viridis* (Cicadellidae), *Philaenus spumarius* (Aphrophoridae), *Aphrophora alni*, *Lepyronia coleoptrata*, *Neophilaenus campestris* and *Stictocephala bisonia* (Membracidae).

*X. fastidiosa* is reported to infect more than 350 different host plants from more than 70 botanical families, some of which are grown in Bulgaria. The possible penetration and spreading of *X. fastidiosa* in the territory of Bulgaria could lead to serious economic consequences as some of the host plants are economically important crops in the country: vine (*Vitis vinifera*), peach (*Prunus persica*), plum (*Prunus domestica* and *Prunus cerasifera*), cherry (*Prunus avium*) and lavender (*Lavandula* sp.). Hosts of the bacterium are also some forest species in Bulgaria, such as maple (*Acer* sp.) and oak (*Quercus* sp.); their infection with *X. fastidiosa* will not manifest direct effect on farming, but will contribute to the overall spread of bacteria in the territory of the country.

At this time, the bacterium is not registered either in the territory of the country or on imported plant material. According to the available data, the yields of certain crops, considered as hosts plants for *X. fastidiosa* (grape – wine and table, plums, cherries, almonds, lavender) are not reduced during the last three years (2015–2017).

### ***Xylella fastidiosa*: spread risk indicators, damaged landscape valuation and socioeconomic impacts**

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**Abstract:** The doctoral research aims to score the risk of the potential entry, establishment and spread of *Xylella fastidiosa* into new countries in Europe and the Mediterranean, and to estimate the economic impacts on olive farms as well as on the landscape in Puglia. In addition, it will simulate the potential economic impact of *X. fastidiosa* if it were established and widespread in Lebanon.

Horizon scanning and econometric models will be used for these purposes. The first conceptual framework will make it possible to calculate an overall risk score for each country, which will be used to rank their risk levels from lowest to highest in terms of entry, establishment and spread of *X. fastidiosa*. On the other hand, the econometric models will profile the impact of *X. fastidiosa* on the communal livelihood of stakeholders

in terms of income source, types of losses, extra costs and reduction in production and landscape value.

### **Empirical assessment of regional vulnerability to *Xylella fastidiosa* based on environmental factors**

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**Abstract:** *Xylella fastidiosa* has already been recorded in numerous areas around Europe. Once the disease has been introduced in an area, its spreading can depend on a number of factors. The biology of the vector, as well as the bacterium itself, in conjunction with the environmental factors such as climate, topography, soil characteristics and land cover will affect the rate of expansion, if left unchecked. This study combines such environmental data from some of the case studies involved in the XF-ACTORS project, with positive identification of *X. fastidiosa*, in order to empirically identify the optimum combinations of values of those parameters. This method assumes no intervention from the authorities or the stakeholders that could prevent the spreading and appearance of the disease in uninfected areas. In addition to the environmental factors, the spatial distance to the nearest positive identification of the disease is also taken into consideration. Subsequently, a vulnerability scale is generated, with areas exhibiting similar combinations of values being classified as highly vulnerable to the bacterium. This scale is applied to the entirety of the regions studied, in an effort to offer a small indication of the likelihood of the disease appearing in currently disease-free areas, where preventive action is mostly needed.

### **Media representations of *Xylella fastidiosa* progress and effects – a cognitive–semantic analysis**

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*The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnte 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

**Abstract:** The imminent threat of *Xylella fastidiosa* has spurred a lot of concern in the affected countries, as well as those where it might yet occur. Here, we analysed the language used to talk about the bacterium using the theoretical framework of the conceptual metaphor theory and critical discourse analysis. More precisely, we aim to discover how *X. fastidiosa*, its spread and effects are framed in public discourse and what the possible effects are of such framing for understanding and controlling the bacterium. The corpus for the analysis comprised 25 texts (totalling 15,153 words) from newspaper and internet sources in English which reported on the bacterium's outbreaks, progress and containment actions in the period from 2015 to 2019. The results indicate that metaphoric conceptualisation focuses on several aspects. First of all, the discourse about *X. fastidiosa* reveals that the bacterium is conceptualised as an entity which is deadly (deadly, lethal, devastating), ominous (it looms, haunts olive oil growers), unpredictable (an unpredictable menace) and unprecedented (an unprecedented disease, a game changing pest, a game changer) or as some other disease (olive tree leprosy, the Ebola of olive trees). Its progress is structured in terms of a journey (it crosses over borders, it reaches the shores, arrives, hitchhikes, it is on a devastating path). Its effects are structured in terms of destruction (it destroys, devastates, wipes out vineyards and olive groves, it wreaks havoc) and killing (it strikes trees, it attacks, it kills, it strangles plant tissues, plants fall victims to it). It is also conceptualised as an enemy (it marches on, it invades the plants) and hence needs to be fought, destroyed, fended off, combated and eradicated. It may be concluded that the most pervasive metaphorical images in media representations related to the bacterium's spread and effects serve to raise awareness of the gravity and magnitude of its occurrence, as well as justify the severe measures undertaken to fight it.

## The Digital Research Object Portal on *Xylella fastidiosa*

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**Abstract:** Opening research data and publishing open access articles have become requirements for many publicly-funded research projects. This is the case of research projects funded through EU Framework Programmes. The number of infrastructures that host open research data has increased to adapt to different types of data, and scientific journals are adapting to the open access policy. The multiplication of open data/open access infrastructure has to be seen positively as they contribute to the visibility, use and re-use of scientific information. Yet, the large number of diverse infrastructures has the counter-effect to scatter information which may be more difficult to reach.

An online publicly accessible portal (Digital Research Object Portal, DROP) has been built in the framework of the XF-ACTORS project. The Digital Research Object Portal works as a node, a unique entry point that facilitates access to digital research objects (data and documents) on *Xylella fastidiosa* stored in different infrastructures. Retrieval of data and documents is possible through the use of keywords that describe the content of the digital objects.

Data and documents produced in the framework of the XF-ACTORS project will be referenced in the next months. These include: genomic data, mass spectra profile data, geospatial datasets, airborne image data, eco-physiological data, open access scientific articles, guidelines, protocols and pictures.

Data and documents produced in the framework of other initiatives, such as the EU-funded project PONTE, the COST Action EuroXanth, the Euphresco project PROMODE and a number of national projects, will be referenced.

If successful, the Portal will be maintained after the end of the project by Euphresco, the network for phytosanitary research coordination and funding and its scope will gradually expand to cover all regulated and emerging pests.

## Environmental risk assessment of *Xylella fastidiosa* subsp. *pauca* in Apulia, based on ecosystem services

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**Abstract:** The entry, spread and establishment of *Xylella fastidiosa* in the olive-producing agroecosystem of Apulia (Italy) affect the flow of ecosystem services and biodiversity in the region. Besides the direct impacts of this invasive pest, control measures against it also cause environmental impacts (e.g. the impact of continuous removal of vegetation or use of insecticides and pesticides on biodiversity; and uprooting of centuries-old trees on cultural heritage). The objective of this study is to assess the short- and long-term negative impacts of *X. fastidiosa* subsp. *pauca* invasion and the control measures against it on the flow of ecosystem services and biodiversity in the olive-producing agroecosystem of Apulia at landscape level, by considering the olive groves and the embedding landscape, which includes other landscape elements than olive groves. Based on the guidance of the European Food Safety Authority (EFSA PLH Panel, 2014) for conducting environmental risk assessments of invasive pests based on ecosystem services, we conducted expert knowledge elicitation: (i) to identify the affected ecosystem services and the associated service-providing units (e.g. species, functional groups, communities, ecosystems) that are responsible for the production and regulation of these services, and (ii) to quantify (in

terms of percentage change) the reductions in affected ecosystem services and the impacts on biodiversity components. Results of the elicitation will be presented.

*Bibliography*  
EFSA PLH Panel

## Session: Sustainable management strategies

### Monitoring viable cells by means of PEMAX-qPCR for screening and evaluation *in planta* of bactericidal compounds against *Xylella fastidiosa*

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**Abstract:** Currently there are no efficient methods to cure *Xylella fastidiosa*-infected plants, therefore methods to monitor the pathogen in plant hosts during the asymptomatic stage of the disease are necessary. Our research deals with the development and evaluation of antimicrobial and defence elicitor peptides against *X. fastidiosa*, and with the analysis of aggressiveness of isolates from different origins in model plant hosts of economic importance. In these studies it is required to know about the physiological state of the pathogen, either in the infected plant or upon interaction with the antimicrobials.

It is well known that the *in vitro* culture of this bacterium presents difficulties due to its low cultivability in synthetic media. Long-term incubation periods (up to six weeks) are required to observe growth. Besides this, and because it is a quarantine pathogen in Europe, strict security measures for working with this bacterium have been imposed for avoiding the risk of spread. Thus, manipulation of cultures and infected plants has to be reduced to a minimum.

In this context, there is a need to develop and optimise molecular methods to detect and quantify viable *X. fastidiosa* cells, as an alternative/complement to plate counting and without the necessity of manipulating cultures or infected plant samples. The viable quantitative PCR (v-qPCR) is a methodology already developed for the quantification of viable foodborne pathogenic microorganisms in different food matrices and for performing viability assessment of biological control strains in the field. This technique constitutes a method that allows the quantification of only viable cells by using nucleic acid-binding dyes such as propidium monoazide (PMA) or ethidium monoazide (EMA) in combination with qPCR. In both cases, the dye intercalates between the DNA of the compromised or non-metabolically active cells, avoiding its amplification afterwards by qPCR.

This methodology that allow to determine viable cells, when combined with conventional qPCR (total *X. fastidiosa* cells), and plate counting (culturable cells), provides information on the physiological states of the bacteria. Thus, it is an advantageous technique for evaluating bactericidal compounds, as well as monitoring the colonisation of *X. fastidiosa* in different host plants during the disease progression.

### Field testing of antimicrobial compounds to mitigate *Xylella fastidiosa* infections in olives

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**Abstract:** The severe impact of *Xylella fastidiosa* subsp. *pauca*, ST53, infections in olives prompted for an intense programme of field testing of different formulations for curative purposes. Under our experimental test conditions several compounds (Fosetyl aluminium; Protein of Harpin; COS-OGA; Acibenzolar S-methyl) proved to be inefficacious to reduce the occurrence and the severity of the desiccation phenomena induced by *X. fastidiosa* on the susceptible olive cultivars. However, among these, applications of N-acetylcysteine (NAC) known to disrupt the biofilm matrix of *Xylella* cell aggregates, and ammonium chloride provided some encouraging attenuation of the symptoms. Briefly, NAC was tested in the field in four different trials under different experimental conditions (trees of different ages, with different initial incidence of symptoms and infections) and modes of application (fertirrigation, soil application mixed with organic fertilisers, trunk injections). Ammonium chloride was initially applied by a local grower by spraying the olive canopies in the attempt to save the olive trees, the evident reduction of symptoms observed prompted then in spring 2019 to set *ad hoc* trials to test different concentrations and number of applications. For NAC, endotherapy applications (one per year) in new plantations (preventive treatments) or in olive groves with only limited initial incidence of the infections, were the only conditions that yielded some reduction in the occurrence of dieback and branch desiccation, even if quantitative PCR on the trees did not show any significant reduction (treated vs non-treated controls) in the bacterial population size. Regardless of the mode of application, the uptake of NAC was confirmed in all cases by HPLC analysis and by the phytotoxicity effects (leaf drop) recorded when the highest doses were used. Similarly, for the olive trees sprayed with ammonium chloride, even if clear symptom reduction was recorded, no differences were recorded on the bacterial populations in the mature tissues of the plants. Along with further observation and tests, the competence of the bacterium to colonise the new growth and its vector-transmissibility from the treated trees (NAC and ammonium chloride) will be assessed.

### **Biological control of the phytopathogenic bacterium *Xylella fastidiosa* by employing bacteriophages with lytic phase**

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**Abstract:** Most of the plant diseases provoking considerable losses in agriculture are caused by bacteria. *Xylella fastidiosa* is a phytopathogenic bacterium living in the xylem, and nowadays is present in many cultivars on our continent, such as avocado, alfalfa, almond, blueberry, coffee, citrus, plum, maple, peach, blackberry, pecan, olive, elm, oak and vine. The dispersion of the bacteria on crops is through sucking insects, such as leafhoppers. The objective of this project is to identify and characterise natural lytic bacteriophages as potential biocontrollers of *X. fastidiosa*.

We hypothesised these bacteriophages could be found in areas of great diversity, and in wine crops where phytopathogenic bacteria have a relationship with this plant; it was also planned to identify potential bacteriophages in water with high microbial diversity. According to our experimental strategy, we isolated, sequenced and characterised specific bacteriophages that infect *Xanthomonas viticola* and *Xylella fastidiosa*. In addition, *in vitro* and greenhouse bioassays have been established to evaluate the biological effectiveness of the use of bacteriophages as an alternative in the management of the disease in the field.

The assembling of the phage genome, genomic annotation and comparison in the database allow us to determine that this is a new phage, not reported in the database. The genome of XV1 is double-stranded DNA of 47,407 bp including 63 open reading frames and an average GC content of 55.56%. *In vitro* bioassays demonstrate the phage ability to infect and lyse *Xanthomonas viticola* and *Xylella fastidiosa*, both pathogenic bacteria of the vine



cultivar. This will allow us to establish management measures for both bacteria in this important cultivar.

### **The Corsican *Xylella* sp. situation evolution: the emergence of an 'inter-worlds' management**

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**Abstract:** As *Xylella* sp. potentially affect around 300 plant species, a large diversity of situations of infestation can occur. Indeed, these plants are potentially situated in a diversity of environments, more or less shaped by human activities, according to socioeconomic interests, land use strategies as well as territorial, social and environmental dynamics. Hence, the management of *Xylella* sp. potentially addresses a multitude of different 'worlds' shaped by stakeholders' activities: agricultural sectors (e.g. olive oil production), forestry exploitation, nature conservation (e.g. Natura 2000 areas), ornamentation sectors (e.g. decorative plants), etc. In this communication, we intend to describe how a form of bio-invasion management emerges and faces the complexity of the different 'worlds' which get connected by the pathogen detection but also by management measures implemented in the territory. Based on a long-term field observation of the *Xylella* sp. situation development in Corsica (participant observation and semi-structured interviews), and relying on Girin's concept of management situations (Girin, 1990), we draw a chronology of management events that stresses the construction of different forms of management situations according to the location of the infected plants. Our study covers a period from winter 2015, where the management situation is framed by the knowledge available on the Puglia situation and seen as an agricultural problem, to winter 2017, where the management situation is framed by a vast part of the Corsica territory infected by *Xylella multiplex*, 28 plant species concerned, and seen as a multidimensional problem. During this period, we show that the management strategy (from preparedness to eradication and then containment), technical procedures, management settings, and of course, knowledge and stakeholder configuration changed, especially some actors' figures such as victims of *Xylella* sp. or whistle blowers (Chateauraynaud & Thorny, 1999). Hence, moving at the interface of different 'worlds', *Xylella* sp. produces a successive set of trials that challenge the management strategies and logic, and the implementation of which finally creates more disturbance in the territory than the pathogen itself.

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Girin

### **LIFE RESILIENCE: prevention of *Xylella fastidiosa* in intensive olive and almond plantations applying productive green farming practices**

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**Abstract:** LIFE RESILIENCE is a European grant with the aim of developing sustainable strategies to reduce the potential spread of *Xylella fastidiosa* (XF) in intensive olive and almond orchards. To do so, LIFE RESILIENCE will demonstrate sustainable best practices and technologies for intensive Mediterranean olive and almond production systems, on 250 ha in Spain, Italy and Portugal. These practices will lower their water consumption and carbon footprint, increase biodiversity and resistance to pests and pathogens without compromising yield. In parallel, LIFE RESILIENCE will also develop new pathogen-resistant and productive olive genotypes as resilient options for olive producers in potentially infected areas, minimising the risk of losses due to XF and other pathogens. The directed crosses between resistant olive cultivars were carried out in 2017 and 2018. The first 500

seedlings coming from these crosses were planted in experimental fields at the end of 2018 for their agronomical evaluation. The plantation followed a randomised block design, with five metres between rows and two metres between trees, using the cultivars Picual, Frantoio, Arbequina and Arbosana as controls. Hundreds of genotypes, which are derived from potentially resistant crossings, will be planted in the same experimental fields during this year. Once selected according to their agronomical traits, the resistance to XF of the best-performing olive genotypes will be tested under controlled as well as field conditions. These potential new olive cultivars can give rise to olive oils with valuable organoleptical qualities, increasing the competitiveness of the sector.

### **Searching for *Xylella fastidiosa* solutions: survey natural enemies of Auchenorrhyncha eggs**

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**Abstract:** The dangerous phytopathogenic bacteria *Xylella fastidiosa* (Wells et al., 1987) has been recently detected in Portugal (January 2019). This disease is transmitted horizontally from infected to non-infected plants by xylem-feeders that belong to the suborder Auchenorrhyncha. The information available until now about natural enemies is reduced. In this work the potential natural enemies of Auchenorrhyncha eggs, the most susceptible stage, were studied during the autumn/winter of 2018/2019. For that, from November 2018 to February 2019 before the egg hatching, on a biweekly basis, 10 samples of 50 g of rests of the remaining vegetation in the ground cover, mainly Poaceae, were collected in an olive grove. In the laboratory, leaves, particularly the interior part of the blade which is a common oviposition location for spittlebugs, were observed under binocular stereoscope. Viable eggs were introduced in petri dishes until hatching. A total of 647 egg-laying masses and 8,222 eggs with a mean of 12.7 eggs per egg laying was recorded. Egg masses were characterised and the action of predation, parasitism and fungi were recorded. Eggs presenting signs of parasitism plus predation were more than 50%. Intact field eggs were kept in controlled conditions until hatching of nymphs and evolution. The parasitoids were identified as *Paracentrobia* sp. (Trichogrammatidae) being the first report for the genus in Portugal. These results constitute an important opportunity to control the main insect vectors of *X. fastidiosa* and containing its spread.

#### *Acknowledgement*

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Wells

### **ResiXO: a project aimed to develop resistant germoplasm for the protection of olive tree heritage in Salento (southern Apulia, Italy)**

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**Abstract:** With the general aim to find sustainable genetic/agronomic solutions for the coexistence of the strategic olive sector with the threat of the *Xylella fastidiosa* epidemic in Apulia, a five-year project, 'Strategies for the containment of olive quick decline: a research and study of resistant germoplasm for the protection of olive tree heritage in Salento (ResiXO)', co-funded in December 2018 by Apulia Region and CNR-Istituto Per la Protezione Sostenibile delle Piante. The Project has two main goals: several resistant

varieties for the new future olive plantations, the protection/survival of the monumental trees substituting the susceptible canopy, by overgrafting, with the resistant cvs. ResiXO, even if enriched by new important experimental activities, inheriting over 13 hectares of experimental fields and pre-select panel of asymptomatic seedlings with interesting phenotypic and technological traits, starts from pre-existing activities, initiated in 2016, of olive germplasm's screening for the identification of new resistant varieties through: the over-grafting of about 450 regional Italian and Mediterranean varieties onto infected mother plants; the selection, in strongly infected areas, of resistant/tolerant spontaneous seedlings. Besides those activities ResiXO aims to verify, study, validate and transfer practical innovations such as: a) new resistant cvs or candidate varieties; b) new information on genomic and metabolic mechanisms of resistance / tolerance; c) new quick and reliable protocols for testing olive germplasm for resistance/tolerance; d) new eco-sustainable strategies for the control of vector populations in infected areas; e) improved over-grafting alternative protocols adapted to specific local conditions and applicable on large scale to save the landscape and the heritage of the monumental olive trees.

### **A systematic large-scale vector monitoring programme to optimise strategies for controlling vectors of *Xylella fastidiosa***

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**Abstract:** Transmission tests carried out in Apulia region (southern Italy) identified three spittlebug species as vector of *Xylella fastidiosa* (Xf) subspecies *pauca*, ST53, causing severe decline on olive trees. This information, along with the data on the host-preference and biology, has been essential to assist the implementation of the containment measures in place, in the so called *Xylella*-demarcated areas, where actions for the control of the vector are mandatory. Since 2019, the Apulian Phytosanitary Authority is supporting a monitoring programme to promote a sustainable and effective application of the vector control strategies (mechanical interventions for reducing the juveniles and applications of insecticides for the adults). The programme aims to provide accurate estimation of the best time to apply the specific interventions, i.e. in relation to the stage of development (juveniles) and to the population dynamics (adults) in olive groves and other crops. A total of 40 representative locations (located at different altitudes) were selected, in the Xf-free area, through the buffer, containment and infected areas. These include cultivated (plots under organic and IPM management) and non-cultivated sites, monitored periodically from March to October. Surveys for juveniles are based on a standard number of field sampling units (transect), whereas the counting of adults is made through traps and a fixed number of sweeping net/canopy/plot. A weekly/biweekly bulletin is then published by the Phytosanitary Authority with recommendations/specific warnings on the most appropriate timing for applying the mandatory control measures. The data so far collected confirmed that the length of the juvenile stages is greatly influenced by the micro-climatic conditions, and as such, the timing for mechanical interventions on the ground vegetation has to be targeted area-by-area. Ultimately, important information is going to be collected regarding the population density, which in the future will help to target the areas/crops/ecosystems where vector control should be prioritised.

### ***Methylobacterium* spp., endophytes of olive trees, as potential biocontrol agents of *Xylella fastidiosa* subsp. *pauca***

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**Abstract:** Interactions between endophytes and plants can promote the health of the host and play a significant role in low-input sustainable agriculture. Understanding this plant-microbe interaction and the mechanisms that enable endophytes to enhance the plant defence response is essential, especially for endophytic bacteria that show biocontrol potential against vascular wilt pathogens. Investigations on the bacterial endophytic population occurring in the xylem of healthy and *Xylella fastidiosa* subsp. *pauca* ST53 (XfpST53)-infected olive trees showed that under field conditions, the population level of cultivable endophytic bacteria is highly variable, being mainly affected by the host genotype, host age, and wilting severity. Among the different cultivable bacteria occurring in the wood of olive trees, *Methylobacterium* spp. are one of the most interesting groups. *Methylobacterium* strains isolated from the xylem of healthy and XfpST53-infected olive trees have been identified as *M. mesophilicum* and *M. radiotolerans*. Species of *Methylobacterium* have also been reported as potential biocontrol agents, plant-growth-promoting bacteria, and resistance inducers, by producing phytohormones, inducing plant systemic resistance, and supplying or mobilising nutritional elements (siderophore production). In order to evaluate the potential of *M. mesophilicum* GR19, and *M. radiotolerans* GR18, GR22 e GR23, as nutrient competitors of XfpST53, the production of siderophores was investigated by using the Chrome Azurol S (CAS) agar and ferric perchlorate assay to detect hydroxamates. *M. mesophilicum* DSM 1708 and *M. radiotolerans* DSM 1819 were used as reference strains. All the tested strains produced different levels of siderophores, and the most effective were applied by endotherapy in healthy and XfpST53-infected olive trees, in order to evaluate *in planta* their activity in containing the olive quick decline syndrome. Moreover, the characterisation of plant-growth-promoting traits of several *Methylobacterium* strains are currently in progress, i.e. by screening the production of indole-3-acetic acid (IAA), and the 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity.