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Introduction

EFSA is hosting the 5th European Conference on *Xylella fastidiosa* from 23 to 25 June 2026 in Mola di Bari (Italy). The event brings together researchers, risk assessors, risk managers and stakeholders to share and discuss the latest scientific and practical advances on *Xylella fastidiosa*, supporting evidence-based risk management and policy decisions.

A series of side events, technical meetings and field activities are organised throughout the week, from 22 to 26 June 2026, offering participants additional opportunities for exchange and hands-on insight into ongoing research and management efforts.

Building on the outcomes of previous editions, the conference aims to present and discuss recent advances in detection, epidemiology and sustainable management of *Xylella fastidiosa*, including social aspects, with particular attention to translating research results into effective risk management and policy support. The event will also address innovative tools and integrated approaches for the sustainable management of *X. fastidiosa* epidemics in olive, vineyard and almond production systems, as well as updates on the risks posed by the bacterium and its insect vectors to agriculture, forestry and natural ecosystems.

The conference is organised by the European Food Safety Authority (EFSA), with the contribution of leading European and international research organisations and networks, including Horizon Europe initiatives such as BeXyl (Beyond *Xylella*), as well as national research projects supporting the control of *X. fastidiosa* and the recovery of affected production systems. The Scientific Committee includes experts from academia, research institutes, regulatory bodies and international organisations. The conference features **eight** thematic sessions (presented in the table below), each chaired by experts in the respective field, and includes contributions from selected abstracts submitted through a dedicated call.

Timing	**22 June 2026**	**23 June 2026**	**24 June 2026**	**25 June 2026**	**26 June 2026**
Morning	Final meeting of the EU-funded Horizon Europe research project BeXyl (Beyond *Xylella*) Organised: by BeXyl project	Opening Session Session 1: Olive resistance to *Xylella fastidiosa*	Session 4: Advances on vectors biology and control Session 5: Impact and social aspects of *Xylella fastidiosa* epidemics and management	Session 8: Pierce's disease of grapevines Closing session	Technical field visits to areas affected by *Xylella fastidiosa* Organised by: Regional Plant Health Service - Puglia Region, CIHEAM Bari, CNR-IPSP.
Afternoon	MASAF projects for the control of *Xylella fastidiosa* and the recovery of the olive growing Organised by: MASAF, CREA	Session 2: Advances on control of *Xylella fastidiosa* Session 3: Advances on biology and control of *Xylella fastidiosa*	Session 6: Monitoring and modelling *Xylella fastidiosa* epidemics 1 Session 7: Monitoring and modelling *Xylella fastidiosa* epidemics 2	Round table "2013-2026: over 12 years of managing the *Xylella fastidiosa* outbreaks – experiences gained and critical issues emerged", Valenzano (BA). Organised by: National Phytosanitary Service (MASAF), Regional Plant Health Service - Puglia Region, CIHEAM Bari, CNR-IPSP.	
Evening		Poster session and Networking reception	Guided tour in Mola di Bari and Social dinner		

A Young Researchers' Initiative is also part of the conference, supporting early-career researchers and plant health professionals through participation in the scientific programme, including oral and poster presentations.

The programme reflects the progress achieved through multidisciplinary research on *Xylella fastidiosa* and addresses scientific approaches to develop, evaluate and implement practical solutions for the control and sustainable management of *X. fastidiosa* outbreaks in the European Union.

Young researchers' initiative

The Young Researchers' Initiative was designed to support participation to the Conference of early-career researchers and plant health professionals, by covering accommodation and travel costs,

In total, 18 young researchers, hailing from Europe and America, have been awarded these travel and accommodation fellowships (8 were funded by EFSA and 10 were funded by Regione Puglia-CIHEAM Bari). To be considered, participants needed to be a presenter of an accepted oral or poster presentation, be either a PhD student or an early career researcher/plant health professional and be affiliated with a public institute or higher education institute.

The awarded young researchers are listed below in alphabetical order by surname:

- Andrea **B**rown (University of California, Berkeley (UC Berkeley), United States)
- François **d**'Alayer (French National Research Institute for Agriculture, Food and Environment (INRAE), BioSP, France)
- Carla Luis **C**arvalho (Institute for Nature Conservation and Forests (ICNF), Portugal)
- Miguel Román **É**cija (Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain)
- Valle **E**gea Cobrero (Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain)
- Fabio **G**atti (Wageningen University & Research, Netherlands)
- Leticia **L**ucero (National Center for Biotechnology (CNB) of the Spanish National Research Council (CSIC), Spain)
- Livia **M**altese (University of Mons, Belgium)
- Luis Alejandro **M**oll Dos Santos (Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain)
- Victor **N**elaton (French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France)
- Elif **O**lkun (Ludwig Maximilian University of Munich (LMU Munich), Germany)
- Elisabetta **P**oeta (Institute of Biosciences and Bioresources (IBBR) of the National Research Council of Italy (CNR), Italy)
- Victória Da **S**ilva (Agronomic Institute of Campinas (IAC) and University of Campinas (UNICAMP), Brazil)
- Giorgia **T**ranchina (University of Palermo, Italy)
- Filipe **V**ieira (Ludwig Maximilian University of Munich (LMU), Germany)
- Francesco **V**olpe (University of Turin, Italy)
- Igor **W**eber (University of Brescia, Italy)
- Jingjing **Y**ang, speaker (Université Catholique de Louvain (UCLouvain), Belgium)

Scientific committee

- **Rodrigo Almeida** (University of California, Berkeley (UC Berkeley), United States)
- **Claude Bragard** (Université Catholique de Louvain (UCLouvain), Belgium)
- **Stephane Compant** (AIT Austrian Institute of Technology, Austria)
- **Daniele Cornara** (University of Bari Aldo Moro, Department of Soil, Plant and Food Sciences (DiSSPA), Bari, Italy)
- **Nik Cunniffe** (Department of Plant Sciences, University of Cambridge, United Kingdom)
- **Ewelina Czwienczek** (European Food Safety Authority (EFSA))
- **Leonardo De La Fuente** (Auburn University, United States)
- **Toufic Elbeaino** (Mediterranean Agronomic Institute of Bari (CIHEAM Bari), Italy)
- **Blanca B. Landa** (Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain)
- **David Makowski** (National Research Institute for Agriculture, Food and Environment (INRAE), France)
- **Maria Saponari** (Institute for Sustainable Plant Protection (IPSP) of the National Research Council of Italy (CNR), Italy)
- **Claudio Sopranzetti** (Oxford University, Oxford, United Kingdom)
- **Giuseppe Stancanelli** (European Food Safety Authority (EFSA))
- **Antonio Vicent Civera** (Valencian Institute for Agricultural Research (IVIA), Spain)

Conference programme

Tuesday 23rd June 2026

09:00-10:00	**REGISTRATION, WELCOME COFFEE & POSTER HANGING**	
	OPENING SESSION	
10:00-10:30	**Welcome and introduction to the conference**	**Giuseppe Stancanelli,** **European Food Safety Authority (IT)**
	Opening remarks by the Regional Councillor for Agriculture, Puglia Region	**Francesco Paolicelli, Regional Councillor for Agriculture, Puglia Region**
	Opening remark by EFSA Executive Director	**Nikolaus Kriz, Executive Director, European Food Safety Authority**
	SESSION 1| Olive resistance to *Xylella fastidiosa* **Chairs: Blanca Landa, IAS CSIC (ES) & Giuseppe Stancanelli, EFSA**	
10:30-10:50 **Keynote**	**1.1 A long-term study on olive cultivars responses to *Xylella fastidiosa* subsp. *pauca* ST53**	**Maria Saponari,** **IPSP CNR (IT)**
10:50-11:00	**1.2 Genomic and molecular insights into olive immunity to Xylella fastidiosa: comparative analysis of Apulian cultivars Ogliarola salentina and Leccino**	**Silke Robatzek,** **Ludwig Maximilian University of Munich (DE)**
11:00-11:10	**1.3 Regeneration of olive crop after the unprecedented epidemic of *Xylella fastidiosa* subsp. *pauca* in Apulia: key factors in the evolving epidemiology**	**Igor Weber*, University of Brescia (IT)** ***Young Researchers' initiative**
11:10-11:20	**1.4 Meta-analysis of a large transcriptomic dataset generated from olive cultivars infected by *Xylella fastidiosa* under different experimental conditions**	**Annalisa Giampetruzzi,** **IPSP CNR (IT) & Pasquale Saldarelli, IPSP CNR (IT)**
11:20-11:30	**1.5 Differential transcriptomic response of three olive cultivars to *Xylella fastidiosa* infection**	**Cinzia Montemurro, University of Bari (IT)**
11:30-11:55 **"Pitch talks"**	**1.6a Top-grafting ancient olive trees as preventive measure against olive quick decline syndrome: preliminary field evidence**	**Pier Federico La Notte, IPSP CNR (IT) & Donato Boscia, IPSP CNR (IT)**

	1.6b The World Olive Germplasm Bank of cordoba: a source for research on *Xylella* control	**Angjelina Belaj,** **Junta de Andalucia (ES)**
	1.6c Genome editing in olive: from embryogenic callus induction to a protoplast-based platform	**Giorgia Sportelli,** **IPSP CNR (IT)**
	1.6d Plant cell wall dynamics in olive cultivars with contrasting responses to *Xylella fastidiosa*	**Daniele Coculo, Sapienza University of Rome (IT)**
	1.6e Integrated metagenomics, metabarcoding and culturomics reveal beneficial bacteria in the xylem microbiome of the resistant olive cultivar Leccino	**Domenico Di Cosmo, University of Bari (IT) & Rim Touati, ISPA CNR (IT)**
11:55-12:30	**Questions and answers**	
12:30-14:00	**LUNCH BREAK & POSTERS hanging**	
SESSION 2 | Advances on control of *Xylella fastidiosa* **Chair: Pasquale Saldarelli, IPSP CNR (IT) and Anna Bonaterra, University of Girona (ES)**		
14:00-14:20 **Keynote**	**2.1 Modes of action and field trials achievements of biopesticides for the control of *Xylella fastidiosa* or its insect vector**	**Stephane Compant, AIT Austrian Institute of Technology (AT) & Pasquale Saldarelli, IPSP CNR (IT)**
14:20-14:30	**2.2 An integrated protocol to mitigate olive quick decline syndrome (OQDS) caused by *Xylella fastidiosa* subsp. *pauca* in southern Italy**	**Giuseppe Lima, University of Molise (IT)**
14:30-14:40	**2.3 Management of olive quick decline syndrome using synthetic bacterial communities**	**Luis Alejandro Moll Dos Santos*,** **IAS CSIC (ES)** *Young Researchers' initiative
14:40-14:50	**2.4 From detection to control: integrating aptamer-based diagnostics with phage and antimicrobial peptide strategies against *Xylella fastidiosa***	**Toufic Elbeaino, CIHEAM Bari (IT)**
14:50-15:00	**2.5 Targeting lipopolysaccharides with synthetic lipopeptides as a control strategy against *Xylella fastidiosa***	**Emilio Montesinos, University of Girona (ES)**

15:00-15:10	**2.6 Evaluation of thermotherapy protocols on nursery plant material for the management of *Xylella fastidiosa* risk**	**Michele Casetta, CRSFA (IT) & Pasquale Venerito, CRSFA (IT)**
15:10- 15:30 **“Pitch talks”**	**2.7a Functional screening of bacterial isolates reveals candidates for the biological control of *Xylella fastidiosa* subsp. fastidiosa in grapevine**	**Luca Nerva, CREA (IT)**
	2.7b Rational discovery of an FtsZ-targeting compound inhibiting in vitro growth across *Xylella fastidiosa* subspecies	**Reinaldo Rodrigues de Souza Neto, IPSP CNR (IT)**
	2.7c Microbial control of *Xylella fastidiosa* through a novel antagonistic protein from the plant phyllosphere	**Filipe Vieira*, Ludwig Maximilian University of Munich (DE)** *Young Researchers’ initiative
	2.7d Nanotrapping *Xylella fastidiosa*: morphophysiological, molecular, and hyperspectral evaluations to uncover mechanistic efficacy and disease reduction potential of cellulose nanocrystal endotherapy.	**Muhammad Amir Muawiya,** **University of Tuscia (IT)**
15:30-16:00	**Questions and answers**	
16:00-16:30	**Coffee/Tea break**	
SESSION 3 | Advances on biology and control of *Xylella fastidiosa* Chair: Stephane Compant, AIT (AT) & Leonardo De la Fuente, Auburn University (US)		
16:30-16:40	**3.1 The xylem endophytome as a driver of disease outcomes in Salicaceae infected by *Xylella fastidiosa* and *Brenneria salicis***	**Lena Pesenti,** **UC Louvain (BE)**
16:40-16:50	**3.2 Potential for blueberry infection by European *Xylella fastidiosa* strains: growth in xylem sap as a proxy for host specificity**	**Leonardo De la Fuente, Auburn University (US)**
16:50-17:00	**3.3 RpfF-mediated DSF production induces priming responses in transgenic *Citrus sinensis***	**Victória Da Silva***, **IAC & UNICAMP, Campinas SP (BR)** *Young Researchers’ initiative
17:00-17:10	**3.4 Prophage diversity among French *Xylella fastidiosa* subsp. *multiplex* strains**	**Marie-Agnes Jacques,** INRAE (FR)

17:10-17:20	**3.5 Phage diversity across *Xylella fastidiosa* subspecies *fastidiosa* and *multiplex***	**Carlos Selles Ribeira, IVIA (ES)**
17:20-17:30	**3.6 Winter recovery of grapevines from *Xylella fastidiosa* infection under mediterranean climate conditions**	**Speaker to be confirmed**
17:30-18:00 **"Pitch talks"**	**3.7a Tracing the origin of the new *Xylella fastidiosa* subsp. *fastidiosa* outbreak in Extremadura, Spain, through comparative genomic analysis and phage typing**	**Felix Mòran**, IVIA (ES)
	3.7b Preliminary transcriptomic profiles of three almond cultivars in response to the infection of different *Xylella fastidiosa* subspecies	**Giorgia Tranchina*, University of Palermo (IT)** *Young Researchers' initiative
	3.7c Exploring the potential of Rep-WH1 prion-like proteins in the control of the phytopathogen *Xylella fastidiosa*	**Leticia Lucero*,** **CNB CSIC (ES)** *Young Researchers' initiative
	3.7d Genetic diversity of *Xylella fastidiosa* in the French region Occitania	**Victor Nelaton*, ANSES (FR)** *Young Researchers' initiative
	3.7e Hfq-dependent small RNAs mediating environmental sensing in *Xylella fastidiosa* support lifestyle transitions and virulence	**Elif Olkun*, Ludwig Maximilian University of Munich (DE)** *Young Researchers' initiative
18:00-18:30	**Questions & answers**	
18:45-21:15	**Networking Reception and Poster session**	

Wednesday 24th June 2026

08:45	**Arrival at venue**	
SESSION 4 | Advances on vectors biology and control **Chairs: Daniele Cornara, Università Aldo Moro, Bari (IT) & Ewelina Czwienczek, EFSA (IT)**		
09:00-09:10	**4.1 BEXYL: Illustrating strategic approaches for entomopathogenic fungi-based Xylella fastidiosa vector management**	**Enrique Quesada Moraga,** **University of Cordoba (ES)**
09:10-09:20	**4.2 Unravelling the interaction between *Xylella fastidiosa* and its insect vectors**	**Daniele Cornara,** **University of Bari (IT)**

09:20-09:30	**4.3 RNA interference (RNAi) for the control of *Philaenus spumarius*, the spittlebug vector of *Xylella fastidiosa* in Europe: lights and shadows**	**Cecilia Parise, CNR-IPSP (IT)**
09:30-09:40	**4.4 Integrated agronomic management targeting the juvenile stages of *Philaenus spumarius***	**Crescenza Dongiovanni, CRSFA (IT)**
09:40-09:50	**4.5 Direct control of insect vectors of *Xylella fastidiosa*: a systematic review and meta-analysis**	**David Makowski, INRAE (FR)**
09:50-10:00	**4.6 Extreme polyphagy in the vector *Philaenus spumarius* — Overview and implications for *Xylella fastidiosa* spread**	**Vinton Thomson, American Museum of Natural History (US)**
10:00-10:10	**4.7 Entomopathogenic Fungi as a Multi-Target Strategy for the Control of *Philaenus spumarius*, the European Vector of *Xylella fastidiosa***	**Francesco Volpe*, University of Turin (IT)** * Young Researchers' initiative
10:10-10:30 **"Pitch talks"**	***4.8a Draeculacephala robinsoni*, a potential vector of *Xylella fastidiosa* in Europe: distribution and population dynamics across the Iberian Peninsula**	**Rocío López Gómez, ICA CSIC (ES)**
	4.8b Metarhizium brunneum* direct exposure reduces survival and disrupts feeding behaviour in *Philaenus spumarius	**Tamara Hernández-Lao, ICA CSIC (ES)**
	4.8c Uncovering viral partners: how the virome shapes fitness in *Philaenus spumarius*	**Pierre Royer, University of Turin (IT)**
	4.8d Assessment of insect vector competence for the acquisition and transmission of *Xylella fastidiosa* to agricultural and forest plant species in Switzerland	**Júlia Lopez-Mercadal, Agroscope (CH)**
10:30-11:00	**Questions and answers**	
11:00-11:30	**Coffee/tea break**	
SESSION 5 | Impact and social aspects of *Xylella fastidiosa* epidemics and management **Chairs: Claudio Sopranzetti, Oxford University (UK) & Anna Maria D'Onghia, CIHEAM Bari (IT):30 – 16:00**		
11:30-11:50 **Keynote**	**5.1 From production decline to structural crisis: olive mill dynamics in a *Xylella fastidiosa*-affected area**	**Pier Paolo Miglietta, University of Salento, (IT)**

11:50-12:00	**5.2 Coexisting with *Xylella*: an anthropological perspective**	**Claudio Sopranzetti, Oxford University (UK)**
12:00-12:10	**5.3 Farmer preferences for Integrated Pest Management strategies against *Xylella fastidiosa*: evidence from Apulia and Alicante**	**Viola Di Cori,** **European Commission Joint Research Centre (ES)**
12:10-12:20	**5.4 Conservation dilemmas: *Xylella fastidiosa* and the future(s) of olive farming**	**Fabio Gatti*, Wageningen University (NL)** *Young Researchers' initiative
12:20-12:30	**5.5 Three years of the EU's 'Plant Health 4 Life' campaign**	**Filippo Positano, European Food Safety Authority**
12:30-12:40 **"Pitch talks"**	**5.6a Mapping research gaps on *Xylella fastidiosa*: toward a socio-ecological perspective for Apulia**	**Livia Maltese* University of Mons (BE)** *Young Researchers' initiative
	5.6b Social dimensions of managing *Xylella fastidiosa*: insights from stakeholders	**Harry Marshall, Forestry Commission Research Agency (UK)**
12:40-13:10	**Questions & answers**	
13:10-14:10	**Lunch break**	
SESSION 6 | Monitoring and modelling *Xylella fastidiosa* epidemics 1 **Chairs: Valerie Olivier, ANSES (FR) & Antonio Vicent, IVIA (ES)**		
14:10-14:20	**6.1 Harmonisation of *Xylella fastidiosa* surveys in the European Union**	**Sybren Vos, European Food Safety Authority**
14:20-14:30	**6.2 The database of 13 years of monitoring in Apulia – over 1,500,000 analysis – a powerful tool for the study of the epidemic of *Xylella fastidiosa***	**Donato Boscia,** **IPSP CNR (IT)**
14:30-14:40	**6.3 Delimiting and managing the *Xylella fastidiosa* outbreak in Alicante (Spain): insights from surveillance and eradication data**	**Martina Cendoya,** **IVIA (ES)**
14:40-14:50	**6.4 Detection of *Xylella fastidiosa* in important Mediterranean plants during dormancy (Euphresco project XfDorm)**	**Anne Laure Boutigny, ANSES (FR)**
14:50-15:00	**6.5 Large scale validation of qPCR assay for simultaneous identification of *Xylella fastidiosa* subspecies in a wide range of plant species naturally infected**	**Giuliana Loconsole, IPSP CNR (IT**

15:00-15:10	**6.6 *Xylella fastidiosa*: from eradication to containment, the Porto metropolitan area (Portugal) case study**	**Carla Luis Carvalho*, ICNF (PT)** *Young Researcher Initiative
15:10-15:20	**6.7 Ten years of *Xylella fastidiosa* in the Balearic islands: impacts on crops and lessons from a multi-subspecies epidemic**	**Diego Olmo ¸IRFAP (ES)**
15:20-15:30 **"Pitch talks"**	**6.8a Rapid volatilomic screening for *Xylella fastidiosa*: towards portable tools for phytosanitary surveillance**	**Elisabetta Poeta,** **IBBR CNR (IT)** * Young Researchers' initiative
	6.8b Horizon Scanning as an integrated approach for monitoring *Xylella fastidiosa*	**Florian Kuntze, European Food Safety Authority**
15:30 – 16:00	**Questions and answers**	
16:00-16:30	**Coffee/Tea break**	
SESSION 7 | Monitoring and modelling *Xylella fastidiosa* epidemics 2 **Chairs: Martina Cendoya, IVIA (ES), & Nik Cunniffe, University of Cambridge, (UK)**		
16:30-16:50 **Keynote**	**7.1 Modelling the effectiveness of *Xylella fastidiosa* monitoring and control across pathosystems and spatial scales**	**Rachel Russell, University of Cambridge (UK)**
16:50-17:00	**7.2 Enhancing *Xylella fastidiosa* spread modelling and surveillance optimisation through remote sensing**	**Rocio Calderon Madrid,** **IAS CSIC (ES)**
17:00-17:10	**7.3 Adapting surveillance design to outbreak characteristics: a dynamic framework**	**François d'Alayer*, INRAE, BioSP (FR)** *Young Researcher Initiative
17:10- 17:20	**7.4 Twelve years of models of *Xylella fastidiosa* epidemics in Europe: lessons learnt**	**Anna Simonetto, University of Brescia (IT)**
17:20-17:30	**7.5 Evolution and consequences of nine years of application of phytosanitary measures to control *Xylella fastidiosa* subsp. *multiplex* outbreak in Alicante**	**Vicente Dalmau Sorlì, Regional Government of Valencia (ES)**
17:30-17:40	**7.6 Integrated surveillance and phytosanitary measures against *Xylella***	**Gianvito Ragone, Regional Plant Health Service, Puglia Region (IT)**

	***fastidiosa* subsp. *fastidiosa* ST1, experience of the Apulia region**	
17:40-17:45 **“Pitch talks”**	**7.7a A hyperspectral phenotyping platform for early detection of *Xylella fastidiosa* infection in woody hosts**	**Valle Egea Cobrero*, IAS CSIC (ES)** * Young Researchers’ initiative
17:45 – 18:15	**Questions & Answers**	

Thursday 25th June 2026

08:45	**Arrival at venue**	
SESSION 8 | Pierce’s disease of grapevines **Chair: Boris Vinatzer, Virginia Tech (US) & Claude Bragard, UCLouvain (BE)**		
09:00-09:20 **Keynote**	**8.1 Pierce's disease of grapevines: climate, diversity, and novel risks**	**Rodrigo Almeida,** **UC Berkeley (US)**
09:20-09:30	**8.2 Impact of simulated climate change conditions on *Xylella fastidiosa*–grapevine interactions**	**Miguel Román Écija*, IAS CSIC (ES)** *Young Researcher Initiative
09:30-09:40	**8.3 Pre-winter pathogen load and cold exposure impact overwinter recovery from Pierce's Disease of grapevine**	**Andrea Brown*,** **UC Berkeley (US)** *Young Researcher Initiative
09:40-09:50	**8.4 Insights and prospects from the Apulian outbreak of *Xylella fastidiosa* subsp. *fastidiosa* in table grapes**	**Serafina Serena Amoia,** **IPSP CNR (IT)**
09:50-10:00	**8.5 Hidden reservoirs of *Xylella fastidiosa* subsp. *multiplex*: prevalence in wild hosts surrounding grapevine regions in the eastern USA**	**Jingjing Yang*,** **UC Louvain (BE)** *Young Researcher Initiative
10:00-10:10	**8.6 Effective cover crop management in vineyards reduces the abundance of *Philaenus spumarius*, the main European vector of *Xylella fastidiosa***	**Clara Lago,** **IFISC-CSIC-UIB (ES)**
10:10-10:20	**8.7 Climate-driven risk assessment of *Xylella fastidiosa* diseases: mechanistic modelling across scales**	**Alex Giménez Romero, CEAB & IFISC CSIC (ES)**

10:20-10:30 **“Pitch talks”**	**8.8a A novel and rapid in vitro method for assessment of *Vitis vinifera* susceptibility to *Xylella fastidiosa* subsp. *fastidiosa***	**Giuseppe Incampo,** **University of Bari (IT)**
	8.8b Introducing the project XYL-o-VINE: Ecology, ethology and transmission biology of *Xylella fastidiosa* by insect vectors in table grape vineyards	**Michela Chiumenti, IPSP CNR (IT)**
10:30-11:00	**Questions & answers**	
11:00-11:30	**Coffee/Tea break**	
CLOSING SESSION : **Blanca Landa, IAS CSIC (ES) & Giuseppe Stancanelli, EFSA (IT)**		
11:30-11:40	**9.1 Regulatory status of *Xylella fastidiosa* in the EU**	**Karin Nienstedt, European Commission, Directorate-General for Health & Food Safety**
11:40-11:50	**9.2 Enhancing Global Plant Health Preparedness for *Xylella fastidiosa* and Emerging Pests: IPPC Tools in Action**	**Sarah Brunel, FAO IPPC**
11:50-12:00	**9.3 Updating the pest risk assessment of *Xylella fastidiosa* for the EU territory: achievements and next steps**	**Matteo Crotta, European Food Safety Authority**
12:00-12:10	**9.4 Feedback from stakeholders: European Nurserystock Association (ENA)**	**Josep M. Pagès, Secretary General, ENA - European Nurserystock Association (BE)**
12:10-12:20	**9.5 Feedback from stakeholders: European farmers and European** **Agri-Ecooperatives association Copa-Cogeca**	**Michele Librandi** **Copa- Cogeca (BE)**
12:20-12:30	**9.6 Introduction to side events:** • **Round table of phytosanitary services at CIHEAM Bari, Valenzano, 25 June 2026 afternoon** • **Field visits in oliviculture and viticulture, 26 June 2026**	**Biagio Di Terlizzi, Director CIHEAM Bari & Salvatore Infantino, Head of Regional Plant Health Service, Puglia Region (IT)**
12:30-13:00	**9.7 Key takeaways from session Chairs**	**Chairs from scientific sessions**

		(One Chair per session reporting back takeaways messages)
13:00	**Conference closing**	

Thematic sessions

Session 1- Olive resistance to *Xylella fastidiosa*

1.1 A long-term study on olive cultivars responses to *Xylella fastidiosa* subsp. *pauca* ST53

Saponari M. (1), Abou Kubaa R. (1), Belaj A. (2), Leon L. (2), Altamura G. (3), Melcarne G. (4), Mousavi S. (5), Mariotti R. (5), Ligorio A.M. (1), Cavalieri V. (1), Dongiovanni E. (3), Saldarelli P. (1), Boscia D. (1)

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Field evaluation of a vast array of cultivars has been a cornerstone of research programs addressing *Xylella fastidiosa* in olives since the 2013 outbreak in Apulia (Italy). In 2015, EFSA funded the first experimental plot (10 olive cvs) in the epidemic area. This screening program, aimed at identifying resistant traits, has since expanded to cover over 200 cultivars. In this report, we analyze molecular and phenotypic data from a subset of 140 cultivars and different Olea europaea subspecies, for which data from over 8 years were available. While diagnostic tests allowed for the differentiation of cvs based on infection frequency after 3–4 years, the long incubation period in young olive trees delayed the collection of consolidated phenotypic data (highly susceptible (HS) controls did not show clear disease manifestation until years 8–10). At that time, 100% of the trees in the HS controls (Cellina di Nardò and Ogliarola Salentina) were infected, with bacterial populations reaching the highest recorded range and symptom scores. In contrast, Leccino trees showed a maximum infection frequency of 41% and a bacterial load two logs lower than the HS controls, with very mild or no symptoms. Based on infection frequencies, consistency of detection over the years and symptom expression, cultivars were categorized into 6 classes, ranging from 1 (HS) to 6 representing the highest level of resistance recorded. Class 1 included 27% of the cvs, class 2 and class 3 included both 22% of the cvs (susceptible and moderately susceptible), with the first showing less pronounced desiccation but still high level of bacterial load; while class 3 included those showing moderate symptoms and intermediate bacterial load. The remaining 3 classes were associated to different level of resistance, as follows: class 4 accounted for 18% of the cvs, including both tolerant and those with intermediate resistance; class 5 included cvs (14%) displaying high frequency of the infections but medium-low bacterial load and very mild or no symptoms; class 6 grouped the cvs (8%) showing low frequency of the infections and bacterial load, and no symptoms. A few cultivars replicated across different plots yielded varying symptom scores, which were more severe in the oldest plots; this suggests that time is a crucial factor in assessing cultivar response. Indeed, differences were observed among the susceptible cultivars, with some still being productive even in presence of desiccations.

1.2 Genomic and molecular insights into olive immunity to *Xylella fastidiosa*: comparative analysis of Apulian cultivars Ogliarola salentina and Leccino

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Olive Quick Decline Syndrome (OQDS), caused by the xylem-dwelling bacterium *Xylella fastidiosa*, poses a severe threat to Mediterranean olive production. Highly susceptible local cultivars such as Ogliarola salentina experience rapid decline and mortality, whereas Leccino displays partial resistance. However, the genetic and immune determinants underlying this resistance remain poorly understood, limiting breeding and management strategies for durable OQDS control. Using advanced sequencing and assembly pipelines for the analysis of the heterozygous genomes of olive cultivars, we generated haplotype-resolved, chromosome-scale genome assemblies for Ogliarola salentina and Leccino. From these genomic resources, we computationally predicted immune receptor repertoires using domain-based annotation, identifying over 1200 pattern recognition receptors (PRRs) and more than 1000 nucleotide-binding leucine-rich repeat receptors (NLRs) across the four haplotypes. Phylogenetic analyses enabled detailed comparisons of PRR and NLR families between cultivars. While both cultivars contain similar numbers of NLRs and receptor-like protein (RLP)-type PRRs, Leccino shows an increased number of class XII leucine rich repeat-receptor like kinase (LRR-RLK)-type PRRs, particularly within a region on chromosome 16. Current work focuses on characterizing these LRR-RLK-type PRRs, functionally testing selected candidates in experimental model plants and monitoring immune responses to defined microbial patterns. In parallel, we analyse transcriptome datasets from trees infected with *X. fastidiosa* to reveal distinct transcriptional signatures in Leccino compared with Ogliarola salentina. Ongoing analyses integrate sequence variation and gene expression to identify immune determinants contributing to resistance.

1.3 Regeneration of olive crop after the unprecedented epidemic of *Xylella fastidiosa* subsp. *pauca* in Apulia: key factors in the evolving epidemiology

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Significant research efforts have focused on understanding the epidemic dynamics of *Xylella fastidiosa* (*Xf*) and on developing integrated strategies to mitigate its spread while supporting the long-term regeneration of olive agroecosystems in Apulia. Within the BeXyl and REACH-XY projects, we investigate the epidemiology of *Xf* in newly established olive groves based on resistant cultivars, under different agronomic and vector management practices. This ongoing experiment, initiated in 2023, included 16 young (5-6 years old) olive groves located in the infected area of Apulia (Southern Italy), combining three factors: (i) cultivar (Leccino, FS17), (ii) soil management targeting vector populations (tillage vs. mulching), and (iii) planting density (low, high and super-high density). Vector populations are assessed through annual nymph surveys along random transects and adult monitoring using chromotropic traps arranged on spatial grids. Vector infection prevalence is assessed in a subset of plots characterized by higher vector density. Olive tree infection status is assessed at the beginning of the study and monitored annually through systematic sampling, allowing the characterization of spatio-temporal dynamics. In addition, eight infected trees per site are monitored to investigate within-canopy infection dynamics. Preliminary results reveal substantial variability in infection prevalence among groves (2–69%), with higher infection levels generally associated with mulching soil, greater vector abundance and the occurrence of abandoned diseased olive groves. This pattern confirms the key role of vector pressure in shaping epidemic trajectories, even in systems based on resistant cultivars. Notably, despite confirmed infections, symptoms were observed only in a single field in 2024. Overall, these preliminary findings indicate that resistant cultivars alone are not sufficient to limit infection spread, as epidemic dynamics remain strongly influenced by vector pressure. The observed variability among groves highlights the importance of integrating cultivar resistance with effective phytosanitary, agronomic, and vector management practices, including the removal of infected trees at the landscape level. Ongoing analyses aim to further disentangle the interactions among management strategies, vector dynamics, and landscape context, and to clarify their role in shaping long-term epidemic trajectories and within-canopy infection patterns.

1.4 Meta-analysis of a large transcriptomic dataset generated from olive cultivars infected by *Xylella fastidiosa* under different experimental conditions

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Xylella fastidiosa is a xylem-limited bacterial pathogen whose impact on major agricultural crops has been documented in the American continent and more recently in EU where olives turned to be particularly susceptible to isolates of the subsp. *pauca*. Understanding the molecular mechanisms underlying host responses to infection is crucial for seeking genetic traits of susceptibility, tolerance and resistance in the vast olive biodiversity. In this study, we performed a meta-analysis integrating transcriptomic dataset from 190 RNA-seq libraries generated across multiple independent projects over nearly a decade of research (2016–present) investigating olive–*X. fastidiosa* interactions. The compiled dataset included transcriptomic profiles from several olive cultivars displaying contrasting responses to infection, including highly susceptible cultivars such as Cellina di Nardò and Ogliarola salentina, and more resistant genotypes such as Leccino and FS17, and Leccino offsprings. Samples were collected under both greenhouse and field infected sources, upon mechanical inoculation or insect-mediated transmission. The dataset also covered multiple infection stages, ranging from early responses (48 hours post inoculation) to advanced stages of bacterial colonization up to 24 months after inoculation. Molecular and phenotypic metadata for each sequenced sample were integrated to provide a robust framework for multi-omic association studies. Our attempts to integrate heterogeneous transcriptomic datasets allowed the identification of conserved transcriptional responses associated with *Xylella* infection, as well as cultivar-specific gene expression patterns modulating levels of the plant stress hormone salicylic acid, potentially linked to resistance and susceptibility mechanisms. These results can provide the basis for the development of a mechanistic model of *Xylella* pathogenesis in olive and of the distinct host responses characterizing susceptible and resistant cultivars.

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1.5 Differential transcriptomic response of three olive cultivars to *Xylella fastidiosa* infection

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Olive Quick Decline Syndrome (OQDS) is a devasting disease responsible for extensive damage to olive groves. Since its first identification in 2013, it has severely impacted the olive sector in the Apulia region, Italy's leading olive oil-producing area. OQDS is caused by the Gram-negative bacterium *Xylella fastidiosa* subsp. *pauca*, which is transmitted by xylem-feeding leafhoppers (Phylaenus spumarious). To date, no effective cure is available, and most olive germplasm is susceptible, with only a few varieties showing resistance, such as 'Leccino', 'Lecciana', 'Leccio del Corno' and 'FS17'. Although several studies have begun to explore the genetic basis of olive responses to *X. fastidiosa*, the molecular mechanisms underlying resistance remain largely unresolved. In this study, we performed a comparative transcriptomic analysis of three olive cultivars with contrasting responses to the pathogen: the resistant 'Leccino', the highly susceptible 'Oliva Rossa', and 'Donna Francesca', which exhibits intermediate susceptibility. Our results revealed distinct and divergent defence strategies among the three cultivars. The susceptible 'Oliva Rossa' underwent extensive transcriptional reprogramming. Despite the strong activation of defence-related responses, including stress-related pathways, secondary metabolism, and cell wall reinforcement mechanisms, these responses appeared largely ineffective in limiting disease progression. In contrast, the resistant 'Leccino' exhibited a more controlled and targeted transcriptional response, characterized by the activation of sulphur assimilation pathways, antioxidant responses, and cell wall–associated proteins, which likely contribute to more effective pathogen containment. Finally, 'Donna Francesca' displayed a comparatively moderate transcriptional response, consistent with its intermediate susceptibility phenotype. Overall, these cultivar-specific transcriptional profiles highlight the complexity of olive–*X. fastidiosa* interactions. Further investigations incorporating a broader range of genotypes will be essential to gain deeper insight into the molecular mechanisms underlying resistance and susceptibility to OQDS.

1.6a Top-grafting ancient olive trees as preventive measure against olive quick decline syndrome: preliminary field evidence

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The resistance traits against *Xylella fastidiosa* subsp. *pauca* in olive cultivars, such as Leccino, have been extensively documented, allowing for the derogation to the ban of planting olives in the demarcated infected area of Apulia region (Italy). The phytosanitary authorities have also authorized top-grafting of ancient/monumental trees using scions of resistant cultivars to reconstruct the canopies and preserve the peculiarity of their trunks. Numerous attempts have been made in the past, both grafting those showing early symptoms and those infected but asymptomatic, resulting either in graft failure or in the development of symptoms after an initial vegetative development. However, when grafting was performed in areas where bacterial infections were not yet widespread, i.e. on non-infected trees, results showed a promising impact in protecting trees from desiccation. In late 2025, we have surveyed and sampled olive trees top-grafted at the time when the majority was not yet infected, but that currently fall in area with high pressure of inoculum. The surveyed trees were top grafted approx. 4 years before, with the canopy fully reconstructed by the vegetation of the resistant cultivar. Visual inspections indicated that these trees compared to non-grafted trees in the surrounding farms had vigorous and healthy-looking canopies. Results of the diagnostic tests, by qPCR assay on shoots collected from the top grafted canopies and, when present, on the shoots sprouted below the grafting points, i.e. from the original susceptible cultivar Ogliarola, positively correlated with visual observations: (i) symptomless grafted canopies tested negative or were found to harbour low amount of bacterial load; (ii) low bacterial populations in the canopies often corresponded to the lack of detection in the shoots collected from the susceptible rootstock of the trees; (iii) canopies showing very mild or more pronounced symptoms usually yielded positive reactions with values indicating high bacterial populations in both the resistant grafted shoots and those grown from the susceptible rootstock. Taken together, these assessments showed that resistant canopies reconstructed on non-infected trees were able to protect the ancient trees from the systemic bacterial invasion thus limiting the impact of the infections. Scions grafted onto infected branches most likely would develop symptoms due to the high pressure of inoculum spreading into the new grafted scions.

1.6b The World Olive Germplasm Bank of cordoba: a source for research on *Xylella* control

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Germplasm banks are essential tools for the conservation, characterisation, and efficient use of olive genetic resources. In this context, the World Olive Germplasm Bank of Cordoba (WOGBC-ESP046), currently the largest olive collection worldwide, comprises approximately 775 accurately identified cultivars and plays a key role in mitigating genetic erosion in olive. Furthermore, the plant material maintained at the WOGBC, together with its study through multilateral and multi-institutional projects, has contributed to generating significant knowledge on species diversity at the morphological, agronomical, molecular, and genomic levels. In addition, the germplasm bank represents a valuable source of diversity to address current and future biotic and abiotic stresses. In the case of *Xylella fastidiosa*, the evaluation of WOGBC cultivars under both controlled and field conditions may contribute to the identification of cultivars resistant or tolerant to the disease caused by this bacterium. At the same time, the identification of promising WOGBC cultivars may provide a genetic basis for the development of new resistant olive varieties through breeding. Within the framework of different projects (XF-ACTORS, E-RTA-Spain, and BEXYL), both controlled and field screening trials involving around 80 cultivars representative of the genetic diversity conserved at the WOGBC have been established in Italy and Mallorca. Besides, the identification by means of EST-SNP markers of olive plants from infected areas of Balearic Islands has also been performed. Additionally, crosses between WOGBC cultivars ('Leccino' × 'Arbosana', 'Leccino' × 'Favolosa') as well as open-pollinated progenies of 'Leccino' and 'Favolosa' have been developed within our breeding programme. Finally, the effect of different thermal treatments on the propagation ability of cuttings of different cultivars of the WOGBC is under evaluation. This with the aim of identifying technical solutions to obtain *Xylella fastidiosa*-free planting material, which is crucial for the exchange of olive plant material for conservation, research, and genetic improvement purposes.

1.6c Genome editing in olive: from embryogenic callus induction to a protoplast-based platform

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Biotechnological strategies in olive (*Olea europaea L.)* are currently under investigation to address the species' recalcitrance to genetic manipulation and in vitro regeneration. In recent years, renewed interest in protoplast-based systems has positioned them as a promising platform for both functional genomics and precision genome editing in olive. However, the capacity of olive protoplasts to re-enter the cell cycle, undergo sustained divisions, and regenerate, ultimately, in whole plants, is limited. Developing effective protocols for olive embryogenic callus induction from adult explants coupled with the integration of genome editing and protoplast regeneration represents a critical bottleneck but also a strategic opportunity for olive improvement; therefore, managing these pivotal steps remains a priority in such species. In this work, different explant types and culture media were tested on three different olive varieties to induce callus formation. Protoplasts isolated from in vitro callus are being used for transient and DNA-free delivery of CRISPR/Cas9, introduced via polyethylene glycol (PEG)-mediated transfection, targeting selected olive genes associated with *Xylella fastidiosa* susceptibility. In parallel, the regenerative potential of olive protoplasts is being examined. Different culture media, osmotic conditions, and growth regulator combinations are being tested to promote continuous proliferation and developmental capability. Preliminary results indicate that the callus induced from adult explants exhibits both embryogenic and regenerative competence. Furthermore, genome editing reagents successfully delivered into protoplasts, suggested that the transfection approach may be feasible. Interestingly, early observations from protoplast regeneration tests revealed the formation of microcalli showing features potentially associated with pre-embryogenic growths. These ongoing experiments are expected to contribute to the research for future genome manipulation and regeneration approaches in this recalcitrant woody species, ultimately supporting efforts to improve many agronomically important traits.

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1.6d Plant cell wall dynamics in olive cultivars with contrasting responses to *Xylella fastidiosa*

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Beyond providing structural support, the plant cell wall (CW) is now widely recognized as an active and dynamic component of plant immunity. Besides acting as a physical barrier against pathogen, the CW can sense and respond to pathogen-induced damage by modulating defense signaling pathways and triggering immune responses (Lionetti et al., 2017; Coculo and Lionetti, 2022; Coculo et al., 2023; Del Corpo et al., 2024). This study investigated the role of the CW in the interaction between the olive tree (*Olea europaea L.*) *and Xylella fastidiosa* (*Xf*), the xylem-limited bacterial pathogen responsible for Olive Quick Decline Syndrome (OQDS), a major threat to olive cultivation in the Mediterranean basin. We compared the composition and remodeling of CW in olive cultivars that displayed contrasting responses to *Xf* infection. Both resistant and susceptible cultivars were analyzed under healthy conditions and during infection to identify CW traits that are constitutive or inducible potentially associated with resistance. Particular attention was given to variations in major CW polysaccharides and to the dynamic remodelling processes occurring during pathogen colonization. Our results reveal specific structural and compositional changes of CW that could be associated with resistance to *Xf*. These findings provide new insights into the possible role of CW remodelling in limiting bacterial colonization and spread within the xylem. We will discuss the implications of these CW dynamics in relation to their potential contribution to resistance mechanisms, as well as their relevance for developing innovative and sustainable strategies to mitigate the impact of this devastating pathogen on Mediterranean olive production systems.

Coculo D, et al 2023. Plant Physiol Biochem 201: 107865

Coculo D, Lionetti V 2022. Front Plant Sci 13: 863892

Del Corpo D, et al 2024. Plant Commun 5: 100931

Lionetti V, et al 2017. Plant Physiol 173: 1844–1863

1.6e Integrated metagenomics, metabarcoding and culturomics reveal beneficial bacteria in the xylem microbiome of the resistant olive cultivar Leccino

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The spread of *Xylella fastidiosa* subsp. *pauca* (*Xfp*) in Southern Italy has caused significant socio-economic damage to olive cultivation. Among existing cultivars, Olea europaea cv. Leccino exhibits a notable degree of resistance. To elucidate the contribution of host-associated microbiota to plant resilience, this study investigated the endophytic microbiota of cv. Leccino and its metabolic functions potentially involved in antagonism toward *Xfp*, which may support new control strategies. A total of 270 trees were sampled across nine orchards in three Apulian regions with varying levels of *Xfp* infection pressure. Xylem tissue and sap were collected during two seasons and subjected to multiple analyses. Shotgun metagenomic sequencing (Illumina technology; ~540 Gb) was analysed using the SqueezeMeta pipeline, which enabled co-assembly, gene prediction, genome binning, and functional annotation. Metabarcoding analysis was conducted on DNA extracted from xylem sap. Concurrently, culture-based isolation of endophytic bacteria was performed to characterize microbial diversity and obtain cultivable strains. A consistent core microbiome was identified. Functional profiling of metagenomic data revealed pathways associated with microbial antagonism, including biosynthesis of antimicrobial compounds, siderophore-mediated resource competition, quorum sensing, and biofilm formation. The integrated analyses identified a set of bacterial taxa with reported plant-beneficial or antagonistic potential that were recurrently detected within the olive xylem microbiome, such as Microbacterium, Sphingomonas, Sphingobium, Novosphingobium, Methylobacterium, Bacillus, Pseudomonas, Arthrobacter, Pantoea, Massilia, and Kineococcus. In total, 82 bacterial species were successfully isolated in culture, establishing a link between sequence-based detection and cultivable strains. Overall, the integration of complementary microbiome-profiling approaches enabled a comprehensive characterization of microbial communities associated with cv. Leccino and identified candidate taxa and functional traits potentially involved in pathogen suppression. These findings provide a foundation for developing synthetic microbial consortia to enhance resistance in susceptible olive cultivars and support the exploration of microbiome-based strategies as sustainable alternatives to conventional plant protection practices.

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Session 2- Advances on control of *Xylella fastidiosa*

2.1 Modes of action and field trials achievements of biopesticides for the control of *Xylella fastidiosa* or its insect vector

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Xylella fastidiosa (*Xf*) is causing diseases in olive and various crops across the Mediterranean region. Due to its rapid transmission, *Xf* is projected to cause yield losses of 35–70% in olive and 13% in almond. Within the BIOVEXO project, 11 partners aimed to develop environmentally sustainable and economically viable solutions to control *Xf* and its insect vector *Philaenus spumarius*. Biopesticides tested included the microorganism *Paraburkholderia phytofirmans* strain PsJN, and plant extracts, based on onion and pelargonic acid (the insecticide Sankari®). In olive, the mechanisms of action of biopesticides include direct biocontrol effects, biostimulant activity, and enhancement of plant immunity. Transcriptome analyses documented changes of olive gene expression after preventive biopesticide applications and early responses to infection. In field trials, persistence and plant colonisation of PsJN as well as the impact of biopesticides on the plant microbiome were investigated. Knowledge of the modes of action will support commercial registration. Onion extract and PsJN were tested under curative trials in olives in the early infection stage, or with widespread infection and severe symptoms. Over four-years, PsJN reduced newly developed and pre-existing symptoms, but only when applied at early infection stages. No beneficial effect was observed in trees with advanced infections and symptoms. Neither formulation resulted in a reduction of *Xf* populations. PsJN mitigated infections in olives, improving stomatal conductance and decreasing canopy temperatures. Preventive trials were established within an IPM strategy combining biopesticides with Sankari® for vector control. In two orchards Sankari® was effective at reducing juvenile populations of *P. spumarius* while adult control employed either Sankari® or the chemical insecticide acetamiprid for comparison. IPM plots treated with Formulated PsJN and with targeted adult vector control showed a marked reduction in the number of infected plants, following applications of either acetamiprid or Sankari®. Results for onion extract were inconsistent: no effect was observed in one trial, whereas in the other a substantial reduction in infection rates was recorded when it was combined with Sankari® against adult vectors. IPM trials produced promising outcomes which require further long-term replicated studies.

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2.2 An integrated protocol to mitigate olive quick decline syndrome (OQDS) caused by *Xylella fastidiosa* subsp. *pauca* in southern Italy

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In this study, we report the effects of selected commercial fertilizers against *Xylella fastidiosa subsp. pauca* (*Xfp*), evaluated both in vitro and in vivo under greenhouse conditions and in an olive orchard of the *Xfp*-infected area. In vitro assays were performed on different *X. fastidiosa* strains, including the *Xfp* ST53 strain responsible for olive quick decline syndrome (OQDS), using the disk diffusion and time-kill assays to determine the bactericidal activity. Greenhouse trials were conducted on potted olive plants of the susceptible cv. Ogliarola Salentina, inoculated by grafting and treated monthly with foliar sprays of the fertilizers. Field trials were carried out over three years on mature olive trees of the susceptible cv. Cellina di Nardò, located in an orchard of the *Xfp*-infected area (province of Lecce, Apulia). In the field, fertilizers were applied within an integrated disease management protocol (IDMP), which included a nutritional plan, agronomic practices (such as light pruning and soil tillage), and *Xfp*-vector control measures. Disease progression was assessed using the McKinney index, and bacterial populations were quantified by qPCR. In vitro results showed that Kalex Zn and Kalex Cu had strong bactericidal activity, completely eliminating *Xfp* cells within 2 to 4 hours of exposure. In greenhouse trials, Kalex Zn significantly reduced disease severity (−50% compared to the control) and decreased bacterial populations (−18.71%). Kalex Cu did not significantly reduce symptom severity but did lower bacterial concentrations (−18.34%) compared to untreated plants. Field trials confirmed these results: treated plants maintained a disease index of approximately 20%, whereas untreated plants reached 61.5%. Moreover, bacterial concentrations were significantly lower in treated plants than in untreated ones. Regarding the olive yield, in the average, treated plants produced 15.7 kg of olives, compared to 7.5 kg in untreated plants. Chemical analysis showed that oil obtained from treated plants met the standards for extra virgin olive oil (EVO) and exhibited increased levels of polyphenols, such as hydroxytyrosol. Overall, our results demonstrate that the tested formulates used within an IDM-strategy not only exert direct antimicrobial effects but also improve olive yield and quality, while enhancing plant resilience to *Xfp*. This approach represents a promising strategy for sustainable coexistence with *Xfp* in infected areas.

2.3 Management of Olive Quick Decline Syndrome using Synthetic Bacterial Communities

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Xylella fastidiosa (*Xf*) is a priority pest in the EU due to its major economic impact on crops such as olive. Currently, no effective curative control measures exist once a plant becomes infected, and deployed management strategies mainly rely on vector control, removal of infected plants, and preventive measures that are only partially effective. These limitations highlight the urgent need for sustainable alternative strategies. In this context, biological control using endophytic microorganisms, particularly Synthetic Bacterial Communities (SynComs) composed of xylem-inhabiting bacteria, represents a promising, environmentally friendly strategy to manage *Xf*-associated diseases. In this study, over 100 bacterial strains isolated from olive xylem were characterized for phenotypic and genetic traits to evaluate their potential as biocontrol agents and/or plant growth promoters. Eighteen biocontrol-related mechanisms were assessed, including antimicrobial enzyme production and plant growth–promoting traits. In addition, carbon source utilization, tolerance to abiotic stresses and in vitro inhibition of *Xf* were evaluated. Complementarily, their genomes were sequenced and analyzed to enable genome-based taxonomic classification and to identify genes associated with biocontrol activity, plant growth promotion, and safety-related traits. Compatibility assays among promising strains led to the design of two SynComs, each composed of three compatible strains. These SynComs were further tested against *Xf* under microfluidic chamber conditions simulating xylem vessels. Their *in planta* efficacy was subsequently evaluated in *Nicotiana benthamiana* and olive plants by assessing disease severity, *Xf* population levels and plant fitness–related parameters (shoot growth, leaf pigment content). Both SynComs significantly reduced disease severity (≈75%) as well as *Xf* populations (1-2.5 log reduction in different plant sections) and improved plant fitness (15-25% increase in height and fresh weight, and ≈38% increase in chlorophyll content) compared with non-treated controls. Field trials are currently ongoing in Italy and Mallorca to evaluate their performance under field conditions.

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2.4 From Detection to Control: Integrating Aptamer-Based Diagnostics with Phage and Antimicrobial Peptide Strategies against *Xylella fastidiosa*

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The development of integrated strategies combining rapid detection with effective and sustainable control is essential for managing *Xylella fastidiosa* (*Xf*). This study presents a unified diagnostic, biocontrol approach based on aptamer technology, bacteriophages, and antimicrobial peptides. High-affinity DNA aptamers targeting *Xf* were generated through 15 rounds of SELEX, followed by counter-SELEX using olive sap-associated bacterial communities to enhance specificity. Three selection strategies; i.e., centrifugation-based partitioning, membrane filtration, and a novel antibody-assisted SELEX, were comparatively evaluated. High-throughput sequencing and AptaSUITE analysis identified enriched candidate pools, with the antibody-assisted approach showing superior performance. Two lead aptamers demonstrated strong specificity and binding affinity, validated by qPCR and Surface Plasmon Resonance (SPR), supporting their application in rapid, field-deployable diagnostic platforms, suhc as SiMoT. In parallel, complementary biocontrol strategies were developed, whilst three virulent bacteriophages (Phi1, Phi3, and MATE2) were isolated and characterized, exhibiting strictly lytic life cycles, high host specificity, and environmental stability. These phages efficiently reduced *Xf* populations in vitro. Additionally, a nisin-based strategy using Lactococcus lactis demonstrated strong antibacterial activity, achieving complete inactivation at low concentrations. In planta assays in *Nicotiana benthamiana* confirmed its effectiveness, with treated plants remaining asymptomatic up to 74 days and evidence of systemic persistence. Together, these results establish a scalable framework integrating early detection with targeted, eco-friendly control strategies, providing a promising basis for next-generation phytosanitary tools.

2.5 Targeting lipopolysaccharides with synthetic lipopeptides as a control strategy against *Xylella fastidiosa*

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Xylella fastidiosa is a xylem-limited Gram-negative bacterium responsible for severe diseases in economically relevant crops, including olive, almond, grapevine and citrus, representing a major phytosanitary threat in the EU. The lack of curative treatment highlights the need for innovative and target-oriented control strategies. Because lipopolysaccharides (LPS) play a key role in the bacterial virulence, colonization, and evasion of plant defenses, synthetic peptides that bind to LPS were evaluated as potential antimicrobial compounds. A set of synthetic peptides, including linear sequences, cyclic peptides and lipopeptides, were screened for LPS-neutralizing capacity using a chromogenic endotoxin assay, and for bactericidal activity against *X. fastidiosa* subsp. *fastidiosa* (ST1). In addition, their hemolytic and phytotoxicity activities were evaluated to define their safety profiles. A significant linear relationship was observed between LPS neutralization and bacterial viability reduction, supporting the role of LPS as a target. Specifically, the lipopeptides incorporating a butanoyl chain displayed the highest LPS interaction. Among them, BP389, BP473 and BP475 combined strong LPS neutralization, high bactericidal activity, and low hemolytic and phytotoxic effects. Confocal microscopy with a fluorescent BP473 derivative confirmed rapid interaction with bacterial cells and membrane disruption, supporting a lytic mechanism like polymyxin-type antibiotics, involving electrostatic binding to lipid A followed by membrane destabilization. *In planta* assays in *Nicotiana benthamiana* demonstrated that, following endotherapy treatment, the peptide moved systemically and significantly reduced both severity symptoms and bacterial populations. These findings identified LPS-targeting lipopeptides as promising candidates to control *X. fastidiosa*. Their dual ability to neutralize a key virulence factor while reducing bacterial viability, along with their low toxicity, supports their further development to be included in integrated plant disease management strategies.

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2.6 Evaluation of thermotherapy protocols on nursery plant material for the management of *Xylella fastidiosa* risk

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Limiting the spread of plant pathogens transmitted through propagation material is an essential requirement to ensure the availability of healthy plants for new plantings. With the spread of the quarantine bacterium *Xylella fastidiosa* in Apulia and other European areas, this need has become even more urgent. In the framework of the EU Horizon Project BEXYL (WP3 Use of thermal treatments for sanitation of plants and production/trade of *Xf*-free nursery materials), starting from the Hot Water Treatment approved for the movement of Vitis propagation material (EU Regulation 2020/1201), different combinations of temperature and exposure time were tested within the framework of the European project BeXyl on several host species, including deciduous species (*Prunus avium*, *P. dulcis*, *Ficus carica*, *Juglans regia*, *Morus alba*) and evergreen species (*Laurus nobilis*, *Lavandula* spp., *Nerium oleander*, *Polygala myrtifolia*), as well as on different types of propagation material (rootstocks, cuttings, and grafted plants). For each condition, agronomic parameters (rooting efficiency, sprouting percentage, shoot length) were evaluated, together with analyses of the effects of TT on plant viability and bacterial load in material confirmed to be infected by *X. fastidiosa*. The results showed a variable response depending on the species and type of material, allowing the identification of initial temperature–time combinations compatible with plant viability and effective in reducing or eliminating the bacterium.

2.7a Functional screening of bacterial isolates reveals candidates for the biological control of *Xylella fastidiosa* subsp. *fastidiosa* in grapevine

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Xylella fastidiosa is a xylem-limited bacterial pathogen threatening several perennial crops, including grapevine, where it can impair water transport and induce severe physiological decline. Although the impact of *X. fastidiosa* in the European viticulture is currently more limited than in olive, increasing global spread and climate-driven expansion raises concerns about its potential establishment in new viticultural areas. Sustainable strategies to contain this pathogen are urgently needed, particularly those compatible with low-input agriculture and capable of preserving beneficial plant–microbe interactions. In this work, we explored the potential of selected bacterial strains as biocontrol agents against *X. fastidiosa* subsp. *fastidiosa* strain Temecula 1, focusing on their ability to interfere its growth and colonization. A first screening phase was conducted in vitro to evaluate antagonistic activity, including inhibition of bacterial proliferation and interference with biofilm formation, a key virulence trait for xylem colonization. Promising biocontrol candidates were then assessed in planta, where their capacity to establish within host tissues and modulate disease progression was investigated under controlled conditions. These experiments allowed the identification of strains capable of reducing pathogen load and/or delaying symptom development, suggesting multiple mechanisms of action, including niche competition and possible induction of host defenses. Overall, our results support the feasibility of exploiting beneficial bacteria to mitigate Pierce's diseases in grapevine, opening perspectives for the development of tailored microbial consortia. As a further step toward broader applicability, we also extended our approach to test selected bacterial strains against *X. fastidiosa* subsp. *pauca*, providing preliminary evidence of their potential activity beyond the initial experimental system and laying the groundwork for future cross-host validation and field-oriented applications.

2.7b Rational discovery of an FtsZ-targeting compound inhibiting in vitro growth across *Xylella fastidiosa* subspecies

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Olive Quick Decline Syndrome (OQDS), caused by *Xylella fastidiosa* subsp. *pauca*, is one of the most severe phytosanitary threats to olive production in the Apulia region of southern Italy. More broadly, *X. fastidiosa* is a xylem-limited bacterial pathogen infecting over 700 plant species, including olive, grapevine, almond, and citrus. Despite its global impact, effective antibacterial compounds against *X. fastidiosa* remain unavailable, underscoring the urgent need for novel control strategies. Filamenting temperature-sensitive Z (FtsZ) is an essential cytoskeletal protein that polymerizes into a Z-ring and coordinates bacterial cytokinesis through assembly of the divisome complex. Phylogenetic analysis of all available genomes showed that FtsZ is highly conserved among subspecies pauca, fastidiosa, and multiplex, differing by only four amino acid residues, supporting its validity as a antibacterial target Building on literature-reported FtsZ inhibitors from other organisms (e.g., S. aureus and E. coli), we selected representative reference compounds and complemented them with a similarity-based selection from a proprietary UniPg small-molecule library using chemoinformatics. The resulting compound set was then submitted to in vitro validation as candidate inhibitors of *X. fastidiosa* FtsZ, and, more broadly, as potential agrochemicals. Transmission electron microscopy revealed impaired cell division in treated cells, characterized by increased cell size. Among 22 structural analogues screened, five significantly inhibited planktonic growth, biofilm formation, and colony-forming units at 100 µM. Dose–response assays (25–200 µM) were performed with the compound 18 which presented high potential inhibiting *X. fastidiosa* growth. This compound inhibited the growth of three major subspecies, reaching 99.99% growth reduction at 200 µM. Preliminary in planta assays in olive plants showed no phytotoxic effects after two months. Overall, these findings validate FtsZ as a conserved and druggable target in *X. fastidiosa* and identify compound 18 as a promising agrochemical candidate for the development of novel antibacterial approaches against this quarantine pathogen. Future studies will assess its efficacy under infection conditions and its potential integration into sustainable disease management strategies.

2.7c Microbial control of *Xylella fastidiosa* through a novel antagonistic protein from the plant phyllosphere

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Plant-associated microbiomes are increasingly recognized as critical determinants of plant health and disease resistance, yet the molecular mechanisms underlying microbial protection against bacterial pathogens remain poorly understood. Here, we identify a bacterial antagonist of *Xylella fastidiosa* (*Xf*), a devastating xylem-limited pathogen responsible for Pierce's Disease in grapevines and other economically important plant diseases. Through systematic screening of 206 Arabidopsis thaliana phyllosphere isolates from the L-SPHERE collection, we discovered that Bacteroidota strain Leaf359 exhibits potent antagonistic activity against *X. fastidiosa* subsp. *fastidiosa* Temecula1 (*Xff* Tem1). We demonstrate that this antagonism is mediated by a secreted 140 kDa hypothetical protein (designated SEL2) that induces bacteriostatic growth arrest and reduces biofilm formation. Structural and functional analysis revealed that the SEL2 N-terminal domain (64 kDa) is both necessary and sufficient for antagonistic activity. Proteomic pull-down assay suggests that SEL2 specifically targets β-barrel outer membrane proteins involved in nutrient transport. Computational modeling suggests a TonB-dependent receptor, thereby inducing nutrient starvation responses in *X. fastidiosa*. Transcriptomic analysis confirmed upregulation of stress response pathways and metabolic genes in SEL2-treated *X. fastidiosa* cultures. Crucially, we demonstrate that a rationally designed low-complexity synthetic bacterial community (SynCom) comprising six SEL2-producing strains significantly suppresses *X. fastidiosa* colonization in A. thaliana plants compared to communities lacking SEL2-producing members. This work elucidates a novel mechanism of microbial control of *X. fastidiosa* and provides a foundation for developing microbiome-based strategies to combat devastating plant bacterial diseases.

2.7d Nanotrapping *Xylella fastidiosa*: Morphophysiological, Molecular, and Hyperspectral Evaluations to Uncover Mechanistic Efficacy and Disease Reduction Potential of Cellulose Nanocrystal Endotherapy

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The widespread Oléiculture losses caused by *Xylella fastidiosa* subsp. *pauca* (strain CoDiRo) underscore the need to shift from external vector management to targeted internal vascular therapies. The present study appraises Cellulose Nanocrystals (CNC), a renewable and biocompatible nanomaterial, as an endotherapeutic agent designed to physically impede pathogen colonization and prevent host physiological deterioration. Extensive in vitro analyses across 0.05% to 1.0% (w/v) CNC concentrations exhibited clear, dose-dependent antimicrobial effects. Automated turbidimetry indicated a markedly extended lag phase and substantial inhibition of bacterial growth at elevated concentrations. Simultaneously, conventional time-kill assays demonstrated an almost complete loss of bacterial culturability. To explain the underlying mechanism, molecular assessment using viability-qPCR (PMAxx) was conducted. The results showed that prolonged exposure to CNC progressively increases definitive bacterial cell death, whereas lower concentrations predominantly induce a Viable but Non-Culturable (VBNC) state. This morphophysiological disruption significantly impairs the complex biofilm structures required for xylem occlusion, thereby acting as an effective vascular nanotrap. To validate these mechanistic insights in vivo, precision stem micro-injections of 1% CNC were performed in a *Nicotiana benthamiana* model. Disease progression and therapeutic effectiveness were assessed using non-destructive, high-throughput hyperspectral phenotyping, which detected subtle stress responses before the appearance of visible symptoms. Untreated, infected plants exhibited rapid and severe physiological decline, including pronounced chlorosis and loss of photosynthetic function. In contrast, pre-symptomatic spectral evaluation of CNC-treated plants indicated comprehensive physiological recovery and a marked reduction in disease severity. Quantitative vegetation indices confirmed the stabilization of photosynthetic efficiency and structural biomass, as well as the effective prevention of tissue necrosis. Additionally, spatial qPCR of stem tissues confirmed that CNC treatment significantly limited the vertical and systemic spread of the pathogen. By restoring the metabolic profiles of infected plants to resemble those of healthy controls, the study validates the efficacy of CNC endotherapy and establishes a scalable, sustainable approach for managing *X. fastidiosa* epidemics.

Session 3- Advances on biology and control of *Xylella fastidiosa*

3.1 The xylem endophytome as a driver of disease outcomes in Salicaceae infected by *Xylella fastidiosa* and *Brenneria salicis*

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Xylella fastidiosa and *Brenneria salicis* are xylem-limited bacterial pathogens responsible for severe vascular diseases in Salicaceae. However, infected plants frequently display contrasting outcomes, ranging from rapid decline to long-term asymptomatic persistence. The mechanisms underlying this variability remain poorly understood. Growing evidence suggests that the xylem-associated endophytic microbiome plays a key role in shaping host–pathogen interactions and disease expression. In this study, we investigate the functional interplay between endogenous xylem bacterial endophytes and these two pathogens, with a particular focus on their capacity to limit pathogen colonization and mitigate xylem dysbiosis. More than 400 cultivable bacterial endophytes were isolated from multiple willow (*Salix* spp.) and poplar (Populus spp.) hosts. These culture-dependent approaches were complemented by targeted metagenomic amplification to refine the taxonomic and functional profiling of the xylem microbiome. In vitro confrontation assays against *X. fastidiosa* strain KLN59.3 and *B. salicis* strain LMG2698 revealed several endophytic isolates exhibiting strong antagonistic activity. Selected strains were further characterized through whole-genome sequencing and phenotypic screening, and genetically transformed with fluorescent markers to enable in planta tracking. Co-inoculation experiments conducted in Salicaceae hosts and *Nicotiana tabacum* model plants provide insights into the spatial and temporal dynamics of endophyte–pathogen interactions within xylem tissues. Overall, our results highlight the xylem core endophytome as a key determinant of disease outcomes in woody plants and support its potential exploitation in sustainable biocontrol strategies targeting xylem-limited bacterial pathogens.

3.2 Potential for blueberry infection by European *Xylella fastidiosa* strains: growth in xylem sap as a proxy for host specificity

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Xylella fastidiosa (*Xf*) causes bacterial leaf scorch (BLS) on southern highbush blueberry (*Vaccinium corymbosum* interspecific hybrids) in the United States, but so far this disease has not been reported outside the US. Twenty-four European and American *Xf* strains were assessed for BLS under greenhouse conditions with the objective of determining if strains isolated from Europe have the potential to infect this host if found in the same region. An intercepted strain from coffee, as well as European strains from grapes, wild olives and lavender caused severe symptoms, while other strains ranged from mildly symptomatic to asymptomatic. We then focused on assessing if blueberry strains can infect grapevine and vice versa. First, growth and biofilm formation in blueberry and grape xylem sap were tested as a proxy for symptoms development and adaptation to these hosts, since virulence assessments in the greenhouse are difficult and lengthy. We complemented the in vitro studies by greenhouse virulence assays in both hosts. We found that in 75% of the strains tested, producing more biofilm when xylem sap was added to the growth medium, correlated with severe symptoms in that host. Although a few exceptions indicate that biofilm-symptoms correlation does not apply to all strains. We then showed in a few selected strains with diverse symptomatology, that the ability to increase growth after serial passages in sap compared to PD3 was a signature of symptomatic strains, while asymptomatic ones died after 3 consecutive 7-day passages. Moreover, we found that most symptomatic strains formed more biofilm when growing on xylem sap collected from susceptible varieties, as compared to sap from tolerant ones. These results suggest that the chemical composition of the xylem sap could influence the outcome of *Xf* infections. Finally, we identified genomic loci putatively involved in blueberry virulence via comparative genomics. Through mutational analysis, a genomic locus containing an orphan RelE toxin that is present only in blueberry-infecting strains, was shown to affect virulence in blueberries. With this study we raise awareness of possible infections of blueberries by *Xf* in Europe and propose in vitro assays and a genetic marker that could be used to predict host adaptation in blueberry and thus help to prepare against epidemics caused by *Xf*.

3.3 RpfF- mediated DSF production induces priming responses in transgenic *Citrus sinensis*

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To mitigate Citrus Variegated Chlorosis (CVC), our research group previously developed transgenic Citrus sinensis plants expressing the rpfF gene (GM), which encodes the RpfF enzyme responsible for synthesizing diffusible signaling factor (DSF), a molecule involved in the quorum sensing system *of Xylella fastidiosa* (*Xf*). DSF overexpression interfered with bacterial signaling, promoting a "pathogen confusion" effect that induces biofilm formation, reduces bacterial motility, and consequently decreases disease severity.To investigate the effect of DSF on plant responses, extracts obtained from GM and non-transgenic control (WT) plants were infiltrated into leaves of *Nicotiana benthamiana*. The results showed a hypersensitive-like response (HR) only at the infiltration sites of hydrophobic extracts from GM plants and synthetic DSF, indicating that the plant is able to perceive these molecules and activate an HR-like defense response. Thus, transgenic plants and their respective WT controls were subjected to transcriptomic and lipidomic analyses. GM plants exhibited induction of several defense-related genes, including pathogenesis-related proteins (PR proteins) such as protease inhibitors (PR-6) and endochitinases (PR-3). Additionally, genes associated with fatty acid and lipid metabolism, such as lipoxygenase 2 (LOX2), were also upregulated. Lipidomic analyses revealed that RpfF interferes with fatty acid metabolism in GM plants, likely competing for substrates involved in the synthesis of DSF-like molecules. Among the detected lipids, lysophosphatidylethanolamine (LPE), a lipid associated with jasmonic acid–dependent signalling, was more abundant in GM plants than in WT plants. Together, these results demonstrate that DSF molecules, in addition to their role in bacterial quorum sensing and in promoting "pathogen confusion" in transgenic plants, may also act as elicitors capable of activating plant defense responses, potentially increasing resistance to other diseases. Together, these results demonstrate that DSF molecules, in addition to their role in bacterial quorum sensing and in promoting "pathogen confusion" in transgenic plants, may also act as elicitors capable of activating plant defense responses. This observation is consistent with previous studies showing that DSF molecules participate in interkingdom signalling, mediating communication between bacteria and their eukaryotic hosts and influencing plant immune responses.

3.4 Prophage diversity among French *Xylella fastidiosa* subsp. *multiplex* strains

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Xylella fastidiosa is a xylem-inhabiting bacterium responsible of relevant crop diseases such as olive quick decline syndrome, citrus variegated chlorosis and Pierce’s disease of grapevine. This pathogen is Native to the Americas and was introduced into France, probably 50 years ago, where four sequence types (STs)—ST6, ST7, ST88, and ST89— of the subspecies multiplex are established in Corsica, PACA and/or Occitanie. Although control measures have limited its spread, the pathogen remains a constant threat to French agriculture, making it essential to understand the drivers of its evolution and dispersal. Bacteriophages shape genetic diversity by promoting gene acquisition, modification and loss, often conferring advantageous traits that enhance host fitness. In *X. fastidiosa*, prophages encode genes involved in key biological functions, including stress tolerance, biofilm formation, and virulence. Given the fundamental role of prophages in bacterial evolution, in this work, we investigated their frequency and diversity in *X. fastidiosa* subsp. *multiplex* strains isolated in France. We analyzed a dataset of 67 complete *X. fastidiosa* subsp. *multiplex* genomes representing all STs reported in France. Bioinformatic analysis revealed a total of 517 prophage sequences, with a median of seven prophages per genome. Further phylogenomic analysis grouped all prophages into 37 species-level clusters belonging to either the class Caudoviricetes or the genus Lophivirus. Both prophage abundance and composition varied significantly across STs and geographical regions, with ST6 strains exhibiting the highest prophage burden. Conversely, strains sharing the same ST and regional origin displayed strong intra-population homogeneity. This pattern supports the hypothesis that multiplex strains in France originated from several independent introduction events. Further comparative studies identified some prophage species-level clusters conserved in multiplex strains globally, suggesting ancestral acquisition events early in the subspecies' evolutionary history. Finally, functional annotation of prophage-encoded proteins revealed genes with putative roles in host colonization such as endoxyloglucan transferases involved in xylem exploitation, suggesting a significant contribution of prophages to *X. fastidiosa* biology.

3.5 Phage diversity across *Xylella fastidiosa* subspecies *fastidiosa* and *multiplex*

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Active outbreaks of *Xylella fastidiosa* in Europe highlight the urgent need for effective and sustainable control strategies. As a xylem-limited plant-pathogenic bacterium responsible for several economically devastating plant diseases, this pathogen remains extremely difficult to eradicate once established. Its persistence in agricultural systems therefore reinforces the importance of environmentally friendly management approaches, including innovative biological tools such as phages, which may provide more targeted and durable control. In this study, we report the isolation and characterization of 42 novel phages capable of infecting European *X. fastidiosa* strains belonging to the subspecies fastidiosa and/or multiplex. Phylogenetic and comparative genomic analyses revealed substantial genomic diversity among the isolated phages and supported the proposal of several new genera and species. The phages clustered into multiple taxonomic groups, with several viral families (such as Autographiviridae, Mesyanzhinoviridae, Casjensviridae, and Tectiviridae) and the subfamily Queuovirinae particularly well represented, highlighting the previously underexplored diversity of *X. fastidiosa*-infecting phages in Europe. Importantly, none of the phages harbored genes associated with lysogeny, virulence, or antibiotic resistance, suggesting a strictly lytic lifestyle and reinforcing their potential as biocontrol agents. Both highly specific and polyvalent phages were recovered, with host range also influenced by the isolation strategy and the bacterial host used. Notably, phages infecting *X. fastidiosa* subsp. *multiplex* ST6 from the Alicante outbreak (mainland Spain) were recovered only by direct isolation using bacterial strains from this outbreak, reflecting a strong preference for *Xylella* hosts over other members of the family Lysobacteriaceae. Some of these phages were also able to infect strains belonging to other sequence types and even to the subspecies fastidiosa. Our results suggest the potential of the novel phages as biocontrol tools, and greenhouse and field trials are currently underway to validate the preventive and therapeutic use of these phages in grapevine and almond.

3.6 Winter recovery of grapevines from *Xylella fastidiosa* infection under mediterranean climate conditions

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Pierce's disease (PD) of grapevine, caused by the bacterium *Xylella fastidiosa*, was first detected in Israel in 2021 and is currently classified as a quarantine disease. The bacterium is transmitted by insect vectors into the plant xylem, where it spreads, disrupts water transport, and ultimately leads to vine decline and death. In California, USA, where the disease has been known for over a century, infected vines have been reported to recover from PD under certain conditions. However, the phenomenon of recovery from PD in grapevine has not been investigated outside the United States. To determine whether recovery also occurs under Mediterranean conditions and to evaluate the effects of cultivar, inoculation timing, and environmental conditions, 350 Cabernet Sauvignon and Chardonnay grapevine seedlings were inoculated with a *X. fastidiosa* isolate obtained from the Upper Galilee region. Inoculations were performed at four time points between May and September. At the onset of dormancy, half of the plants remained at the Volcani Institute (central Israel), while the other half were transferred to overwinter at the Upper Galilee. Before the second growing season, all vines were pruned and returned to the Volcani Institute for continued growth. Vines were monitored over two consecutive growing seasons, and each plant was assessed for infection through both visual symptom evaluation and molecular detection. During the first season, infection rates in vines inoculated in May reached 80–95%, with a similarly high proportion of symptomatic plants. In contrast, vines inoculated in late September exhibited no visible disease symptoms, although approximately 60% of the plants were infected. In the following season, 100% of vines inoculated in late September recovered from the infection, whereas only about 15% of vines inoculated in May recovered. Neither dormancy location nor cultivar had a significant effect on infection or recovery rates. Overall, the results suggest that early-season infection allows the bacterium to establish more effectively within the plant, including colonization of woody tissues, leading to chronic infection that persists even after pruning. In contrast, late-season infection appears to be transient and allows plants to recover from the disease following winter dormancy and pruning. These findings will help define the critical infection window in vineyards and contribute to the development of management strategies for PD in Israel.

3.7a Tracing the origin of the new *Xylella fastidiosa* subsp. *fastidiosa* outbreak in Extremadura, Spain, through comparative genomic analysis and phage typing

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Xylella fastidiosa is a quarantine bacterial plant pathogen that poses a major threat to European agriculture due to its wide host range and capacity to cause severe diseases in economically important crops. In 2024, a new outbreak of *X. fastidiosa* subsp. *fastidiosa* (*Xff*) was detected in Extremadura (Spain), near the Portuguese border, raising concerns about its possible origin and regional spread. This study aimed to characterize the strains associated with this outbreak using molecular detection, phage typing, and comparative genomic analysis based on core-genome. A total of 45 samples, including plant material and potential insect vectors, were collected during official surveys conducted in high-risk areas. Molecular detection using EPPO-recommended real-time PCR assays confirmed the presence of *Xff* in 18 plant samples belonging to several Mediterranean shrub species (Lavandula, Cistus, Cytisus, Calicotome, Spartium, and Pterospartum), as well as in Vitis vinifera. The pathogen was also detected in one specimen of the known vector Philaenus spumarius. Multilocus sequence typing (MLST) revealed that all positive samples corresponded to sequence type ST-1. Five representative isolates were successfully cultured and subjected to whole-genome sequencing using a hybrid strategy combining Oxford Nanopore long reads and Illumina short reads, enabling the reconstruction of complete genomes. Comparative genomic analyses, including ANI, pangenome reconstruction, and SNP-based phylogeny, were performed together with a global dataset of representative *X. fastidiosa* genomes. In parallel, phage typing using five bacteriophages revealed a distinct lytic profile for the Extremadura isolates, clearly differentiating them from previously characterized strains from Mallorca and reference strains from the United States. Genomic analyses also revealed, for the first time, a plasmid insertion associated with *Xff* strains, suggesting a potential role of mobile genetic elements in the genetic differentiation of the outbreak isolates. Together, these results provide the first genomic characterization of the *X. fastidiosa* outbreak in Extremadura and contribute to understanding the genetic diversity and epidemiology of *Xff* in Europe. The integration of genomic epidemiology with phage typing represents a promising approach for tracing outbreak origins and improving surveillance and management strategies for this high-impact plant pathogen.

3.7b Preliminary transcriptomic profiles of three almond cultivars in response to the infection of different *Xylella fastidiosa* subspecies

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Xylella fastidiosa is a detrimental xylem-limited bacterium responsible for severe diseases in a wide range of plant species, including almond (*Prunus dulcis*), where it causes the almond leaf scorch. In this work, we aimed at investigating the early infection responses in different combinations cultivar-bacterial strains to characterize those combinations showing susceptibility or resistance. RNA-seq libraries were built from xylem tissues recovered from mature shoots of the cultivars Tuono, Avijor (Lauranne) and Pizzuta d'Avola collected 48 hours after mechanical inoculation with *Xylella fastidiosa* strains of the subspecies *pauca*, *fastidiosa* and *multiplex*. Transcriptomic data were integrated with SNP genotyping analysis obtained from an Axiom™ 60K almond SNP Array from the three cultivars in order to provide a genomic framework for interpreting transcriptional variation. A distinct expression profile among inoculated cultivars was observed, indicating a genotype-dependent response to Xylella fastidiosa infection. Furthermore, the SNP analysis enabled the detection of a list of unique polymorphisms, useful to increase the specificity of the cultivar response to the pathogen. An ad hoc bioinformatic pipeline was developed to jointly analyze the two datasets, enabling the identification of candidate genes and/or molecular pathways potentially involved in early defense mechanisms, as well as genetic variants that may underlie the contrasting responses observed among almond cultivars. Overall, this integrative approach could provide robust insights on the mechanisms activated in some almond cultivars to counteract bacterial infection. Future perspective will include the selection of the main candidate gene targets, their validation by quantitative RT-PCR and further functional studies useful in breeding strategies.

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3.7c Exploring the potential of Rep-WH1 prion-like proteins in the control of the phytopathogen *Xylella fastidiosa*

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Xylella fastidiosa is a bacterial phytopathogen that proliferates in the xylem of the infected plants to the point of occluding their vases, thus leading to the irreversible drying of plants. We have explored novel approaches to combat *X. fastidiosa*, since there is no effective solution for the infected plants. The characterization of the Rep-WH1 prion-like proteins was carried out through the integration of microbiological (both in Escherichia coli and *X. fastidiosa*) and molecular biology methods, advanced optical microscopy, TEM, biophysics. We have found orthologues of the Rep-WH1 prion-like domain of the Pseudomonas plasmid pPS10 in the replication proteins of plasmids in *X. fastidiosa*. The expression of two of these Rep-WH1 prion-like proteins decreased the viability of E. coli and X. fastidiosa cultured in vitro. In *E. coli*, the pXF51 prion-like protein causes the conversion of the bacillary cells into cocci. Expression of the Rep-WH1 prion-like proteins decreased the virulence of *X. fastidiosa* on experimental infection of *Nicotiana benthamiana* plants, compared with the control bacteria, as revealed by net reductions in the symptoms of withering, in the bacterial load (qPCR) and by an anomalous senescent morphology of bacteria within the xylem vases (TEM of sections across plant petioles). In E. coli, by means of fluorescence recovery after photobleaching (FRAP), I have found that the pXF51 *X. fastidiosa* prion-like protein undergoes a phase transition from solubility to amyloid. These results open a way to the possible exploitation of these prion-like proteins for the control of this phytopathogen.

3.7d Genetic diversity of *Xylella fastidiosa* in the French region Occitania

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Xylella fastidiosa is a plant pathogenic bacterium which presence in Europe has been known since its first detection in Italy in 2013. Since then, various studies have shown that multiple introductions from the American continent have occurred in Italy, France, Portugal and Spain leading to the establishment of several distinct populations belonging to three subspecies and multiple Sequence Types (ST) currently present in these four countries. The Occitania region in France is the most recent of the three French regions where the bacterium has been detected. The study aims to provide a better understanding of the diversity, epidemiology and pathogenicity of *Xylella fastidiosa* in Occitania in order to support decision-makers implementation of effective surveillance policies in France. A Multilocus Variable number tandem-repeat Analysis (MLVA) has been conducted on 331 samples, including infected plant material, isolated bacterial strains and insect vectors, to explore the genetic diversity of *X. fastidiosa* in Occitania. Our results show that this region is mainly infested by *Xylella fastidiosa* subsp. *multiplex*, ST6. Within this population, two genetic subgroups can be distinguished using several statistical methods such as Discriminant Analysis of Principal Components, or STRUCTURE®, suggesting either an on-site differentiation, or two independent introduction events. These two subgroups show no clear or simple geographic distribution pattern. Several ST7 strains and corresponding plant samples were also found out within a relatively restricted area. These strains were likely introduced from a nearby French region as their MLVA haplotypes are closely related. Genome-based approaches will allow us to further explore the *Xylella fastidiosa* dynamics in Occitania. In addition, pathogenicity tests and statistical analysis of surveillance data will be conducted. These approaches should help to improve our understanding of the bacterium's overall population dynamics and to provide relevant information for decision-makers in the context of outbreaks management.

3.7e Hfq-dependent small RNAs mediating environmental sensing in *Xylella fastidiosa* support lifestyle transitions and virulence.

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One of the infection strategies of *Xylella fastidiosa* (*Xf*) is the ability to switch between biofilm formation and planktonic growth state in response to environmental cues, which is mediated by quorum sensing (QS) and associated with increased production of extracellular vesicles (*Xf*-EVs). Our profiling of *Xf*-EVs revealed the presence of RNA-binding proteins, in particular, the RNA chaperone Hfq, along with non-coding small RNAs (sRNAs), indicating their potential role in mediating these lifestyle changes via *Xf*-EVs. Hfq and sRNAs are known to regulate virulence and transition between biofilm and planktonic state in other pathogens, however their role in *Xf* is largely unknown. To investigate this, we generated a Δhfq mutant of *Xylella fastidiosa* subsp. *fastidiosa* Temecula 1 (*Xff*), which results in a non-motile, aggregated state, reduced motility, and impairment in its ability to systemically colonize host plants such as grapevine and model organisms- Arabidopsis thaliana and Nicotiana tabacum. To further understand the regulatory role of Hfq and to identify its associated sRNAs, we did transcriptomic profiling and sRNA sequencing of *Xff* WT and Δhfq in rich and xylem mimicking media conditions. While *Xff* WT shows a dynamic response to different media conditions, this response is impaired in Δhfq, particularly in genes and transcriptional regulators associated with twitching motility, biofilm regulation, QS, and virulence. sRNA sequencing of both strains revealed reduced sRNA profile of Δhfq, pointing to their dependency to Hfq and their role in post-transcriptional regulation of important genes. RIL-Seq experiments will further identify Hfq-dependent sRNAs and their specific mRNA targets that are crucial for *Xf* response to environmental changes and virulence. Overall, our findings suggest Hfq as a key regulatory mechanism for environmental adaptation and virulence in *Xff*.

Session 4- Advances on vectors biology and control

4.1 BEXYL: Illustrating strategic approaches for entomopathogenic fungi-based *Xylella fastidiosa* vector management

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The European research project BEXYL funded under the Horizon Europe programme (2022-2026), has provided important evidence on the key role of entomopathogenic ascomycetes in the management of *Xylella fastidiosa* (*Xf*) vectors. Due to their distinctive contact integumentary mode of action within entomopathogens, these fungi are among the very few alternatives for the microbial control of piercing-sucking spittlebugs. In addition, the intriguing relationships they form with plants are notable as they facilitate diverse strategic applications aimed at reducing spittlebug nymph or adult populations and at preventing potential transmission of the Olive Quick Decline Syndrome (OQDS), driven by *Xf*. BEXYL has demonstrated that entomopathogenic fungi can be employed both for their direct effects as microbial control agents of *Xf* vectors and for indirect effects mediated through interactions with olive crops and associated weeds. Several species of entomopathogenic fungi can control *Xf* nymphs either through direct application to olive ground cover or indirectly via endophytic colonization or priming of cover crop and weed communities. In addition, these fungal biocontrol agents may also be deployed to target adult *Xf* vectors, both through direct fungal applications aimed at adult populations within olive orchards and, more importantly, by exploiting the endophytic or rhizosphere competence of entomopathogenic ascomycetes within the olive crop, which may reduce adult fitness and limit bacterial transmission potential. Finally, the potential collateral impact on *Xf* vectors resulting from applications of entomopathogenic ascomycetes against other olive pests should also be considered.

4.2 Unraveling the interaction between *Xylella fastidiosa* and its insect vectors

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Insect vectors play a central role in the spread of several plant pathogens, with pathogens often impacting vector physiology and behavior. *Xylella fastidiosa* is a propagative and persistent, yet non-circulative, xylem-limited bacterium that is transmitted by xylem sap–feeding insects such as spittlebugs and sharpshooter leafhoppers. Upon acquisition, *X. fastidiosa* colonizes the chitinous cuticular lining of the insect foregut, where bacterial cells adhere, multiply, form biofilms, and eventually detach from. This biofilm reduces the diameter of the food canal (precibarium in particular) through which sap is ingested, likely making feeding more energetically costly. In addition, bacterial cells can exploit chitin as a carbon source mediated by a chitinase. The reduction of diameter in the food canal, the potential exploitation of the insect foregut as a nutrient source, and the effects of *X. fastidiosa* infection of vectors on probing behavior, has led to the hypothesis that *X. fastidiosa* may exert detrimental or pathogenic effects on its vectors. However, clear experimental evidence supporting this hypothesis is still lacking. Here, we investigated the impact of *X. fastidiosa* foregut colonization on the spittlebug vector *Philaenus spumarius* through an integrative approach combining Electrical Penetration Graph (EPG) recordings of probing behavior, respirometry-based measurements of metabolic rate, lipid reserve analysis, and mRNA-seq–based differential gene expression. Our results indicate that the presence of the bacterium on the insect foregut significantly affects feeding activity, muscle function, energy metabolism, and immune response. Overall, we observed a suppression of innate immune cascades, including the Imd (immune deficiency) pathway and melanization. Consistent with declined metabolic rate and reduced fatty acid amounts in *X. fastidiosa*-infected individuals, transcriptomic analyses revealed a metabolic shift toward lipid catabolism in insects harboring the bacterium. Moreover, differential expression of genes associated with muscle contraction and salivary gland activity was detected in *X. fastidiosa*–positive insects. These changes may be linked to partial obstruction of the foregut lumen caused by bacterial colonization, which likely increases the energetic demand required to power the cibarial pump and sustain feeding. EPG data showed that *X. fastidiosa*-positive insects experienced greater difficulty initiating xylem sap ingestion, with this impairment positively correlated with bacterial load in the foregut. However, once feeding was initiated, ingestion bouts tended to last longer in infected insects, possibly compensating for the reduced frequency of ingestion events. Overall, our findings demonstrate that *X. fastidiosa* colonization of the insect foregut impose physiological costs to its vector, which appear to be mitigated through compensatory, energy-demanding mechanisms. Although long-term population-level effects were not assessed, these results support the hypothesis that the bacterium exert negative effects on insect vectors, potentially influencing vector survival and reproductive fitness.

4.3 RNA interference (RNAi) for the control of *Philaenus spumarius*, the spittlebug vector of *Xylella fastidiosa* in Europe: lights and shadows

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RNA interference (RNAi) has emerged as one of the most promising non-chemical strategies for pest control and crop improvement. Described in most eukaryotes, it is defined as a natural regulatory and defense mechanism against viruses and transposons. RNAi can be harnessed to silence essential genes required for insect survival through the delivery of sequence-specific double-stranded RNA (dsRNA). For successful RNAi application, two critical parameters must be considered: first, the target pest must possess the core genes necessary for the RNAi machinery to function properly; and second, the selection of an appropriate target gene is essential. Furthermore, the field application of RNAi remains hindered by additional challenges, the most widely discussed of which is the efficiency of dsRNA delivery. While gene silencing has been explored in various hemipteran species, including aphids, psyllids, sharpshooters, and leafhoppers, no investigations had previously involved the meadow spittlebug, *Philaenus spumarius* (L.) (Hemiptera: Aphrophoridae). Over the past three years, in the frame of the SOS project funded by the Italian Ministry of Agriculture, MASAF, we investigated the efficacy of RNAi in *P. spumarius*. We first characterized the core RNAi machinery genes and confirmed the mechanism's functionality in both nymphs and adults. Subsequently, a high-throughput screening of possible target genes was conducted to identify candidates inducing sterility or high mortality. Our findings highlighted two key targets: ATP synthase beta and proton-ATPase. Silencing ATP synthase beta resulted in complete female infertility, characterized by the absence of eggs in the ovaries of females microinjected with specific dsRNA. On the other hand, proton-ATPase knockdown significantly increased mortality and reduced the bacterial load within the insect gut during *X. fastidiosa* transmission assays. While dsRNA delivery was primarily optimized via microinjection, plant-mediated feeding (by petiole absorption and hydroponic) also proved to be effective in both nymphs and adults. Nevertheless, an effective method of delivering dsRNA to this insect via feeding, avoiding genetic transformation of the plant, has yet to be developed. By targeting fitness and fecundity of *P. spumarius*, RNAi represents a promising approach for a highly specific and sustainable control of the vector and of its transmission ability.

4.4 Integrated agronomic management targeting the juvenile stages of *Philaenus spumarius*

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In Apulia, *Philaenus spumarius* is the main vector of *Xylella fastidiosa* due to its wide distribution and high transmission efficiency. In infected areas, vector control remains the most effective strategy to limit pathogen spread, making the management of juvenile stages a key priority, as their low mobility and close association with ground vegetation allow early population suppression. Over the past three years, field trials were carried out in Apulia (southern Italy) as part of the BeXyl project to assess different strategies for managing juvenile stages in olive groves. Small-scale trials (80 m^2 per treatment) tested cover crops (Cruciferae, Fabaceae, and Poaceae, alone or in mixtures), natural substances (nonanoic acid and maltodextrin), and entomopathogenic fungi under various management conditions, compared with spontaneous vegetation and conventional soil practices such as tillage or mulching. Simultaneously, large-scale trials (each covering 1 hectare) were established in sixteen intensive and super-high-density olive orchards with resistant cultivars (FS17 and Leccino), managed through tillage or mowing, to monitor juvenile and adult vector population dynamics. Alternative strategies reduced nymphal populations by 40–70%, offering viable options where mechanical methods are limited by environmental constraints. However, large-scale trials showed that soil management was more effective, with tillage more effective than mowing. These benefits were sustained only with consistent management in the surrounding area; otherwise, recolonization by adults from nearby fields offset the benefit of juvenile control. Overall, agronomic practices play a key role in spittlebug management, highlighting the need for a territorial-wide coordination. While soil tillage remains the most effective approach, alternative methods can be integrated where mechanical control is not feasible, supporting a flexible and sustainable IPM strategy.

4.5 Direct control of insect vectors of *Xylella fastidiosa*: a systematic review and meta-analysis

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Since its detection in Europe in 2013, *Xylella fastidiosa* has been reported across Mediterranean regions, posing a major phytosanitary threat to agriculture, landscapes and biodiversity. In the EU, the meadow spittlebug, *Philaenus spumarius*, is the main vector, although other xylem-feeding insects are competent vectors. Effective vector control is central to disease management. This EFSA Scientific Opinion evaluates direct vector control measures through a global systematic literature review, critical appraisal and quantitative meta-analysis. The search covered all known xylem-feeding insect species and control interventions, including studies published up to January 2026. Of 1253 records retrieved, 77 publications met the criteria for critical appraisal and 57 were included in a meta-analysis estimating vector survival reduction across different types of control measures. Based on published data, the statistical analysis compared synthetic active substances (approved and non-approved in the EU as of 10 December 2025) with alternative approaches, including entomopathogenic fungi, entomopathogenic nematodes, non-synthetic substances and predators. Synthetic substances currently approved in the EU showed the highest vector survival reduction. Cyantraniliprole, deltamethrin, acetamiprid and lambda-cyhalothrin demonstrated the greatest estimated efficacy, although their ranking remains uncertain due to limited data and heterogeneous experimental conditions. Non-approved synthetic substances generally showed lower efficacy, while alternative approaches produced more uncertain effects. Key limitations of published trials include variability in study design, limited field validation and insufficient long-term assessment. Recommendations are proposed to improve standardisation and reporting, strengthening the evidence base for sustainable and integrated vector management of *Xylella fastidiosa* in the EU.

4.6 Exreme polyphagy in the vector *Philaenus spumarius* — Overview and implications for *Xylella fastidiosa* spread

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Philaenus spumarius (L.), the meadow spittlebug, primary vector of *Xylella fastidiosa* in Europe, is an extreme polyphage, making it all the more dangerous as a vector. We have compiled all recorded P. spumarius hosts, using two major sources, a deep review of the published literature and data from the BRIGIT citizen science project, in which UK residents submitted photographic host records. The initial result was a world record 1,311 host plants, now expanded to over 1,400 hosts by subsequent addition of published records. This presentation will review biological patterns of host plant use, including special attraction to nitrogen-fixing and ectomycorrhizal plants, and the implications of host use patterns for spittlebug spread of *X. fastidiosa*.

4.7 Entomopathogenic Fungi as a Multi-Target Strategy for the Control of *Philaenus spumarius,* the European Vector of *Xylella fastidiosa*

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The meadow spittlebug *Philaenus spumarius* is the primary European vector of *Xylella fastidiosa* and plays a central role in the epidemiology of Olive Quick Decline Syndrome in Apulia (southern Italy), where *X. fastidiosa* subsp. *pauca* has caused the death of more than 10 million olive trees. Vector control currently involves spring tillage targeting juveniles in the herbaceous cover and insecticide applications against adults. However, these measures often provide only temporary suppression due to recolonization of treated areas during the season and raise significant concerns regarding environmental impact, non-target effects, and economic sustainability, highlighting the need for alternative management strategies. Entomopathogenic fungi (EPF) represent a promising tool for sustainable pest management, combining direct pathogenicity against insect vectors with potential indirect effects when acting as endophytic entomopathogenic fungi (EEF). EEF can colonize plant tissues and induce systemic resistance through secondary metabolites or immune priming, potentially affecting pest development and survival. This study aimed to: (i) screen 20 EPF strains for insecticidal activity against juvenile stages of *P. spumarius*; (ii) assess the efficacy of the most promising strains against adults; (iii) test the most promising EPF strain under field condition in Apulia; iv) Evaluate the endophytic potential of selected EPF strains. The results are promising; several EPF strains belonging to the genus Metarhizium showed high direct pathogenicity with a mortality rate of up to 98% for nymphs and up to 75% for adults. In particular, the strain M. anisopliae Bp1q was used in field trials in Apulia and significantly reduced vector abundance in olive groves, supporting its potential inclusion in an integrated vector management program. The EPF strains M. anisopliae Cnv2i, *Clonostachys rosea* Cas1h and *Purpureocillium lilacinum* CB2 showed high endophytic colonization efficiency. Future research should further investigate the effects of EEF on different aspects of *Philaenus spumarius* biology, and the mechanisms underlying their plant colonization and multitrophic interactions (EEF–plant–vector). Such advances will be essential to consolidate strengthen the application of EPF as microbiological control agents against all developmental stages of *P. spumarius*, improving the vector management with a multi-target strategy.

4.8a *Draeculacephala robinsoni*, a potential vector of *Xylella fastidiosa* in Europe: Distribution and population dynamics across the Iberian Peninsula

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The recent introduction in Europe of *Draeculacephala robinsoni* Hamilton (1967), a potential vector of the phytopathogenic bacterium *Xylella fastidiosa* (*Xf*), represents a new threat to key crops in Europe. Although its role in pathogen transmission has not yet been investigated, its detection in 2021 in northeastern Spain has prompted studies on its biology, ecology, and behaviour. Systematic field surveys were conducted during 2024 and 2025 in Spain, more precisely Catalonia, Galicia, Valencia, and La Rioja. Two sampling methods were employed: sweep net and D-VAC aspiration. Surveys targeted habitats susceptible to colonization, including permanent pastures, cereal and alfalfa fields, ground cover vegetation and canopy of woody crops such as vineyards, apple orchards, and olive groves. To date, *D. robinsoni* specimens have been recorded exclusively in Girona and northern Barcelona province in Catalonia, where the species occurs year-round, with no evidence of obligatory diapause, up to four generations/year. In 2024, four population peaks were identified in May, June, August, and, most prominently, October. Similarly, four peaks were detected in 2025; however, phenology shifted, with peaks in April, July, a major peak in September, and October. In both years, the highest population densities were observed in rice fields and permanent pastures dominated by perennial ryegrass (Castelló d'Empúries, Girona). Intermediate densities were recorded in alfalfa and grass mixtures (Castelló d'Empúries), with no significant differences between years. Population density on ground cover vegetation in olive groves, apple orchards (Sant Pere Pescador, Girona), and vineyards was lower, but *D. robinsoni* adults were also present on the vine canopy in Fortià (Girona). In 2025, the results were supported by captures obtained from yellow sticky traps placed on the woody crops sampled, and on the Fraxinus edges of perennial ryegrass fields. Captures were recorded from September to October, coinciding with population peaks. These results indicate that populations are well-established across all surveyed crops, reflecting the ongoing spread of the insect throughout Catalonia, expanding up to Barcelona province. These results highlight the need to continue field surveys to establish the geographical expansion of *D. robinsoni* in order to better understand its ecological requirements and its potential role in *Xf* spread in mediterranean agroecosystems.

4.8b *Metarhizium brunneum* direct exposure reduces survival and disrupts feeding behaviour in *Philaenus spumarius*

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The meadow spittlebug, *Philaenus spumarius* (*Ps*), is the main European vector of *Xylella fastidiosa* (*Xf*), a xylem-limited bacterium causing severe diseases in key crops such as olive, almond and grapevine. Current management strategies mainly focus on vector control, often through habitat manipulation. However, approaches targeting vector feeding behaviour remain largely unexplored. The use of entomopathogenic fungi represents a complementary approach, not only by reducing vector survival but also by potentially altering insect feeding behaviour before death. Previous studies evaluated the indirect plant-mediated exposure of Ps to *Metarhizium brunneum* (*Mb*) effects on feeding behaviour, suggesting a potential role in *Xf* management. In this study, we assessed the effects of the direct application of Mb strain EAMa 01/58-Su and subsequent changes in their feeding behaviour. *Ps* adults were directly exposed to a suspension of Mb strain and maintained for 72 hours on untreated Sonchus oleraceus plants to avoid indirect plant-mediated fungal effects. Mortality was higher in *M. brunneum* challenged insects, reaching a 75.0% ratio, while the control mortality was 8.7%. Insect feeding behaviour on *S. oleraceus* and olive plants was monitored using the electrical penetration graph (EPG) technique, quantifying the number and duration of key events associated with *Xf* transmission, such as probing, xylem contact (Xc), xylem ingestion (Xi), resting, and scape events. In addition, Ps mortality was recorded daily for ten days, and fungal colonization of Ps was confirmed through microbiological analysis. Preliminary results indicate that on S. oleraceus, Mb-infected insects reached Xc and initiated Xi significantly faster than controls (7.5±1.1 vs. 20.4±3 and 7.8±1.1 vs. 20.7±3.9, respectively), whereas infected individuals exhibited longer total resting time than controls (34.7±7.5 vs. 20.9±7.1). On olive plants, *Mb*-infected insects showed significantly longer Xi than controls. Given its lethal effects on the vector, Mb could be a key tool to manage its populations and could be integrated with agroecological practices such as ground-cover management to finally enhance disease suppression while reducing reliance on chemical insecticides. Future research should evaluate *Mb* under field or semi-field conditions, particularly on plants hosting established Ps populations, to better assess its potential within integrated *Xf* management strategies.

4.8c Uncovering viral partners: how the virome shapes fitness in *Philaenus spumarius*

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Within agroecosystems, research has predominantly addressed plant viruses and their insect vectors, leaving viruses pathogenic to insects comparatively unexplored. Apart from the extensively studied Baculoviridae family, whose members are widely used as biocontrol agents against Lepidoptera, little is known about other insect-infecting viruses and their host interactions. Expanding our understanding of virus–host interactions in insect orders beyond Lepidoptera, such as Hemiptera, may provide valuable insights for the development of novel biocontrol strategies. In this study, we focused on viruses of the spittlebug *Philaenus spumarius* L. (Hemiptera: Aphrophoridae), the main European vector of the bacterium *Xylella fastidiosa*, the causal agent of Olive Quick Decline Syndrome in the Apulia Region of Italy. The virome of four populations of *P. spumarius*, from Chieri, Arnasco, Bellino (North-West Italy) and Sault (South-East France) was characterized, and virome–host interactions were evaluated. Five fitness-related parameters were assessed: adult longevity and fecundity, larval hatching rate, development time and survival. As the Arnasco population was found to be almost virus-free, a virus-infected isogenic population was generated by micro-injecting purified viruses into Arnasco males and females. Fitness parameters estimation is ongoing in their progeny, thereby avoiding any potential effects of the micro-injection in the parental generation. This experimental design allows us to compare the effects of the virome on host fitness between virus-free and virus-infected individuals of an isogenic population. Furthermore, these results are compared with those obtained with the three other populations, characterized by different viral community composition. This study provides a first insight on virome–host interactions of *P. spumarius*; further attention is given to the transmission modality of these viruses to characterize their horizontal and vertical pathways.

4.8d Assessment of insect vector competence for the acquisition and transmission of *Xylella fastidiosa* to agricultural and forest plant species in Switzerland

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Xylella fastidiosa is a highly damaging plant pathogen with significant economic, ecological, and social implications. Native to the Americas and already established in southern Europe, it is categorized as a priority quarantine organism in Switzerland and the EU. To proactively evaluate the risk of its spread in Switzerland, this project is aimed to assess the capability of a selection of insect species commonly present in Switzerland to acquire and transmit *X. fastidiosa* (several subspecies) to woody plants of agricultural and sylvicultural interest. Acquisition and transmission experiments were conducted in biosecurity level 3 facilities at WSL (Birmensdorf, Switzerland) with insect vectors *Philaenus spumarius*, *Aphrophora alni* and *Cicadella viridis*. The targeted plants included agricultural species (*Vitis vinifera*, *Prunus armeniaca*) and sylvicultural species (*Fagus sylvatica*, *Quercus robur*) of interest for the Swiss landscape, as well as shrub species (*Rubus fruticosus*, *Corylus avellana*) that bridge and occur in both ecosystems. Two types of acquisition systems have been tested; namely from artificially inoculated plants and from artificial diet. Acquisition rates from source plants were 0 % for *P. spumarius*, 0.8 % for *A. alni*, and 1.7 % for C. viridis. Acquisition from artificial diet increased to 28.3 % for *A. alni* and 13.1 % for *C. viridis*. Transmission occurred only when bacteria were acquired from artificial diet, reaching 50 % for *A. alni* on *Q. robur* and for *C. viridis* on *R. fruticosus*. Host-plant susceptibility varied seasonally: infection rates were 50–100 % for all plants in July, but in November only *Q. robur*, *F. sylvatica*, and *C. avellana* remained infected at 50–100 %. Given the late-season (November) sampling, reduced detection in some hosts may result from bacterial retreat into the plant tissues. These results provide crucial insights into the epidemiology of *X. fastidiosa*, identifying potential pathways for its spread across Swiss agricultural and forest ecosystems. Moreover, these findings will support its early detection by improving targeted surveillance, contribute to containing its propagation and, ultimately, limit the economic losses in cultivated crops and the impacts on forest ecosystem services.

Session 5- Impact and social aspects of *Xylella fastidiosa* epidemics and management

5.1 From production decline to structural crisis: olive mill dynamics in a *Xylella fastidiosa*-affected area

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The study provides a spatiotemporal statistical assessment of the structural crisis affecting the olive oil processing sector in the Salento area (Province of Lecce, Italy) over the period 2014–2015 to 2025–2026. Using administrative and production data, the analysis investigates trends in the number of olive mills, their territorial distribution, and key supply-chain variables, including quantities of olives processed and olive oil produced. Results show a marked and persistent decline in the number of active mills, decreasing from 232 units in 2014–2015 to 111 in 2025–2026, with a clear structural break emerging from the 2018–2019 production season onward. Spatial analysis at the municipal level highlights a widespread and severe mortality pattern, with most municipalities experiencing losses exceeding 75%, indicating a systemic rather than localized phenomenon. Parallel trends are observed in production variables: both olives processed and oil produced exhibit a substantial contraction over time, particularly after 2018–2019. The decline affects all processing typologies (own-account and third-party), with third-party processing and purchased olives remaining the dominant components of the system. Strong positive correlations among all variables suggest a highly integrated production system, where shocks propagate across the entire supply chain. The observed dynamics are consistent with a significant reduction in raw material availability and processing capacity, largely attributable to the spread of *Xylella fastidiosa* in the region. The pathogen’s impact appears to have triggered cascading effects on the economic sustainability of olive mills, contributing to their progressive closure. These findings underline the need for targeted policy interventions aimed at supporting sector resilience, fostering productive reconversion, and integrating phytosanitary, economic, and territorial strategies. Future research should focus on quantitative spatiotemporal modelling to disentangle the specific contribution of *Xylella fastidiosa* subspecies *pauca* relative to other environmental and market drivers, enhancing evidence-based management of affected agro-ecosystems.

5.2 Coexisting with *Xylella*: An Anthropological Perspective

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This paper offers an anthropological reflection on coexistence with *Xylella fastidiosa* in Salento, focusing on the post-epidemic phase in areas where the bacterium has become endemic and has led to the biological or “productive death” of millions of olive trees. It departs from this latter category—a condition in which the tree still displays vegetative signs but is no longer economically sustainable—in order to explore how different conceptions of the tree and its function generate divergent models of agronomic, ecological, and landscape management. After briefly outlining the epidemiological trajectory of *Xylella* in Apulia since 2013 and its economic and labor consequences, the analysis turns to the infected zone, where emergency has given way to coexistence. Three main models have emerged. The first is widespread abandonment, shaped by land fragmentation, hobby farming, high costs of uprooting and replanting, and policies primarily directed at professional farmers. The second is uprooting and replanting, particularly through tolerant cultivars in intensive and super-intensive systems, or through crop conversion. The third is the maintenance of affected trees through continuous pruning, soil management, treatments, and, in some cases, grafting. Drawing on interviews and ethnographic cases, the presentation shows how these models are rooted in distinct visions of what a tree is and what determines its death. For some, the tree is primarily a means of production, a unit of capital to be mechanized and replaced when no longer profitable. For others, it is a historical, environmental, and landscape heritage to be preserved even at economic loss. In still other contexts, it becomes an ornamental element or a “calling card” within a tourist economy. Within these perspectives, productive death may or may not coincide with biological death—not because of physiological differences, but because of the function attributed to the tree. The central argument is that these ideas are not merely abstract or emotional positions, but materially consequential factors shaping practices, responses to scientific analysis, acceptance or rejection of institutional directives, and the reconfiguration of the landscape. Ignoring this plurality produces generalized policies unable to adapt to a territory in which the same tree may represent different things to different actors—and even to the same person. Taking these differences seriously is therefore essential for understanding.

5.3 Farmer preferences for Integrated Pest Management strategies against *Xylella fastidiosa*: evidence from Apulia and Alicante

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Xylella fastidiosa remains a major threat in Mediterranean agricultural systems, where containment policies rely heavily on mandatory vector control and phytosanitary measures under the Commission Implementing Regulation (EU) 2020/1201. In demarcated areas such as Salento (Apulia, Italy) and Alicante (Spain), growers are required to implement insecticide applications and soil tillage to reduce vector populations. Within the Horizon Europe BEXYL project, this study evaluates farmer preferences for alternative Integrated Pest Management (IPM) strategies that aim to reduce pesticide dependence while maintaining effective disease control. We implement a Discrete Choice Experiment (DCE) with at 400 olive growers in Apulia and 200 almond growers in Alicante. Growers choose between three IPM programs -one focusing on vector control, one focusing on increased plant resistance, and one being a combination of both - and a status quo reflecting current mandatory practices. the alternative choices are characterized by: (i) biological control measures; (ii) plant-based resistance measures; (iii) reduction of insecticide use; (iv) subsidy level (€/ha); and (v) minimum collective adoption thresholds required for effective control, to assess farmers' responses to collective action requirements. Preferences will be estimated using Random Parameters Logit models to account for unobserved heterogeneity. Welfare measures will be derived as willingness to accept (WTA) compensation for implementing IPM attributes and compensating surplus for each program. The study provides evidence on the trade-offs growers face between biological control, pesticide reduction, subsidy incentives, and peer adoption constraints. Results will inform the design of socially acceptable and economically viable IPM policies in *Xylella*-affected areas, supporting EFSA's objectives on sustainable and integrated disease management.

5.4 Conservation dilemmas: *Xylella fastidiosa* and the future(s) of olive farming

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Over the past decade, *Xylella fastidiosa* has profoundly transformed olive-growing landscapes in the southern Italian region of Apulia. Once characterised by ancient olive trees that sustained both rural livelihoods and cultural identities, the region is now caught between two competing policy trajectories: on the one hand, efforts to restructure and intensify olive production in order to maintain economic viability and competitiveness; on the other hand, attempts to preserve centuries-old olive trees valued as cultural heritage and key elements of biocultural landscapes, despite their high management costs. This presentation conceptualises this tension as a conservation dilemma: the structural difficulty of sustaining olive trees simultaneously as productive crops, sources of income, and components of socially and culturally valued landscapes. Drawing on long-term ethnographic research and political economy analysis of disease management measures, rural development instruments, and farming responses, the paper examines how different strategies for managing the impacts of *X. fastidiosa* are linked to different visions of agricultural futures. These strategies are enabled—or constrained—by specific policy frameworks, subsidy schemes, market conditions, and labour arrangements. By situating plant health governance within broader questions of rural sustainability and landscape management, the presentation argues that the long-term future of Apulian olive groves depends not only on technical responses to the pathogen, but also on the policy and economic conditions that shape which practices of care can effectively be sustained over time. The paper concludes by discussing the need for plant health governance approaches that are not only scientifically robust, but also attentive to the socio-economic and cultural conditions shaping the feasibility, legitimacy, and long-term sustainability of disease management measures.

5.5 Three years of the EU's 'Plant Health 4 Life' campaign

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Since 2023, the European Food Safety Authority (EFSA) has been running the #PlantHealth4Life campaign (PLH4L) together with the European Commission and participating countries across and beyond the EU. By 2025, the initiative attracted 30 participating countries, and thanks to this growing community, the campaign has now been extended to continue in 2026, with unprecedent participation from 33 countries. This multiyear campaign aims to raise public awareness of the risks associated with plant health and their impact on food security, biodiversity, and the environment. PLH4L focuses on three audience segments identified as both at higher risk and influential within their communities: i) curious travellers, ii) home gardeners and hobby farmers, and iii) conscious young parents. The campaign encourages these groups to travel and shop responsibly, and to talk to their children about plant health, underscoring the important role that every citizen has in protecting plant health. The campaign was launched in 2023 with the objective of raising awareness, introducing the topic of plant health to the audience. In 2024, the second year of the campaign, the focus shifted towards critical thinking, encouraging citizens to consider plant health in their everyday decisions. The third year focused on driving audience engagement, encouraging informed citizens to become messengers of the campaign. The campaign includes a tailored national implementation featuring a variety of communication activities, such as in-person events (e.g. fairs, exhibitions, and school events), family-oriented materials (e.g. the mascot Pesty and a colouring book for children), and additional activities (e.g. out-of-home advertising, social media, and media partnerships). Monitoring shows that the campaign has helped to increase awareness of the impact of plant health on the economy and food safety among those who saw it. Eight in ten citizens now acknowledge that plant health is connected to their daily lives.

Relevance to *Xylella*:

The campaign's emphasis on responsible plant movement and public vigilance is particularly pertinent in preventing outbreaks of *Xylella fastidiosa*, one of the world's most dangerous plant pathogens which requires strict prevention measures and early action measures to avoid further spread.

5.6a Mapping Research Gaps on *Xylella fastidiosa*: Toward a Socio-Ecological Perspective for Apulia

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Since the discovery of *Xylella fastidiosa* subsp. *pauca* as the etiological agent of Olive Quick Decline Syndrome (OQDS) in Apulia, research and funding have been directed toward a set of urgent questions: How can the bacterium be effectively detected? What is the ecology and behaviour of its vector? How does the pathogen interact with the host plant? And ultimately, how can its spread be contained? More than a decade after the first detection, substantial progress has been made, yet the bacterium continues to expand slowly northward, with new subspecies and sequence types still being identified. This study conducts a systematic review of existing literature to evaluate how scientific attention has been distributed across different research domains and to identify gaps in current knowledge. The analysis classifies publications into six categories: (1) pathology, (2) vector ecology and spread modelling, (3) diagnostics and monitoring, (4) management and control measures, (5) ecological drivers of bacterial spread, and (6) socio-economic impact analysis. Results reveal that approximately 40% of the literature focuses on the vector–host–pathogen relationship, 38% on monitoring and eradication strategies, while only 14% adopt broader socio-ecological perspectives whit ecological drivers of the bacterial spread mostly overlapping with the study of vector ecology. These findings suggest the need to strengthen research on the social and ecological dimensions of the epidemic. Understanding the agroecological conditions that enabled the emergence and persistence of *Xylella* in Apulia could help identify alternative management scenarios that reduce disease incidence and landscape vulnerability. Likewise, exploring social dynamics may clarify the polarized reactions and conflicts that have shaped local responses. By establishing bridges across disciplines and stakeholder groups, this study advocates for a more collaborative and co-creative approach to addressing the complex challenge of *Xylella fastidiosa*.

5.6b Social dimensions of managing *Xylella fastidiosa*: insights from stakeholders

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Xylella fastidiosa (*Xf*) continues to generate profound economic, ecological, and social disruption across Europe. While biological and epidemiological knowledge has advanced rapidly, effective preparedness and management also rely on understanding the perceptions, behaviours, and constraints of those directly involved in surveillance, mitigation, and long-term adaptation. Within the EU-funded BeXyl project, we conducted comparative qualitative research to investigate how different stakeholder groups understand and respond to *Xf* risk across three contrasting European contexts: Córdoba, Spain (*Xf*-free); Alicante, Spain (*Xf* detected in 2019); and Salento, Italy (*Xf* established since 2013). These regions differ markedly in vector pressures, host crop systems, disease history, and regulatory management mandates (monitoring, eradication, containment), offering a unique natural experiment to explore the social dimensions of preparedness and response. Using a mixed-methods approach, we carried out 21 interviews and 14 focus groups with farmers, agronomists, cooperative representatives, and other key actors (N=146). Findings reveal strong context-dependence in risk perception, trust, and evaluations of management measures. In Córdoba, perceived vulnerability was low and knowledge limited. Stakeholders in Alicante expressed high concern, frustration with eradication mandates, and uncertainty about future impacts. A decade into the epidemic, participants in Salento demonstrated a gradual shift toward pragmatic adaptation, including interest in resistant varieties, diversification strategies, and long-term coexistence approaches. Across all regions, prevention and monitoring were broadly supported, yet concerns were consistently raised regarding feasibility, cost, technological complexity, and governance. These insights underscore the need for region-specific, socially informed integrated pest management (IPM) strategies and highlight the importance of embedding behavioural and socio-political factors within preparedness frameworks for vector-borne plant diseases.

Session 6- Monitoring and modelling *Xylella fastidiosa* epidemics 1

6.1 Harmonisation of *Xylella fastidiosa* surveys in the European Union

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The harmonisation of pest surveys across the European Union is vital for robust, targeted plant health monitoring. This oral presentation will centre on the design of surveys for *Xylella fastidiosa*, detailing the various survey types—detection, delimiting, and buffer zone surveys—and their respective roles in early warning and containment strategies. Emphasis will be placed on the Plant Health Law and associated legislative requirements which provide the regulatory backbone for these activities. The EFSA Pest Survey Toolkit will be showcased as a cornerstone for achieving harmonised and standardised survey approaches, highlighting practical elements such as pest survey cards and databases essential for survey preparation. Attendees will be introduced to advanced tools including RiPEST and RiBESS+, which streamline survey design by specifying input parameters and output (survey efforts). RISKER will be discussed for its effectiveness in implementing risk-based surveys, while OPTIPEST offers comprehensive solutions for multipest surveys at the crop level. The harmonised survey report generated by RiPEST will be presented in the context of the EUROPHYT Survey reporting obligations. The presentation will conclude with a practical illustration, demonstrating the application of the toolkit within a virtual *X. fastidiosa* detection survey scenario. Participants will gain insight into the technical processes and operational tools that underpin harmonised *X. fastidiosa* survey throughout the EU.

6.2 The database of 13 years of monitoring in Apulia – over 1,500,000 analysis – a powerful tool for the study of the epidemic of *Xylella fastidiosa*

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EU legislation on measures to prevent the introduction and spread within the Union of *Xylella fastidiosa* requires each Member State to conduct annual monitoring for territorial surveillance and, where the bacterium is detected, to implement eradication or containment measures. In line with this framework, the Apulia Region has implemented a monitoring program, still ongoing, to support surveillance, and, where appropriate, to implement eradication or containment actions. From 2013 through the end of the 2025 monitoring campaign, a total of 1,511,773 plants were sampled and analyzed. This effort, remarkable for both the number of samples and laboratory tests performed, is unique internationally. Although the scale of the work makes it difficult to replicate and sustain in the long term, it has significantly advanced understanding of the spread and complexity of *Xylella fastidiosa*. The Regional Plant Protection Service of Apulia deemed it appropriate, also for reasons of transparency, to make such a large dataset accessible to both the scientific community and the general public through the institutional website www.emergenzaxylella.it of the Apulia Region, which also provides maps and cartographic representations for each annual monitoring campaign. The purpose of monitoring is not to inventory infected plants, but to anticipate the bacterium by identifying new outbreaks at an early stage, thereby reducing inoculum pressure on uninfected areas and slowing, as far as possible, the expansion of the infected zone. In addition to underpinning containment actions, the data and their cartographic representation make it possible to visualize the pathogen's progression, from the initially rapid diffusion in southern Salento to a slower advance towards the north. The database also provides additional valuable insights, including: i) evidence of different degrees of susceptibility among host species, which has enabled the phytosanitary authority to introduce certain derogations from the ban on planting host species in infected areas; and ii) further confirmation of the etiological role of subsp. *pauca* as the agent of Olive Quick Decline Syndrome (OQDS).

6.3 Delimiting and managing the *Xylella fastidiosa* outbreak in Alicante (Spain): insights from surveillance and eradication data

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In Alicante (Spain), *Xylella fastidiosa* subsp. *multiplex* (*Xf*) was first detected in 2017, affecting mostly almond, although to date it has been identified in 29 plant species. In accordance with EU regulations, official surveys and outbreak management measures have been implemented since the first detection. However, it remains unclear whether newly detected *Xf* positives indicate pathogen spread or simply reflect previously undetected infections in poorly surveyed areas. Official surveillance data collected annually between 2017 and 2024, consisting of georeferenced plant samples (both positive and negative) and records of completed tree felling activities, were used to explore the spatio-temporal distribution of *Xf* within the outbreak area. For each year, the distance between the newly detected *Xf* positives and the nearest eradicated area was calculated. Simulations were performed by generating random points within the surveyed area each year, matching the number newly detected *Xf* positives. The distances from these randomly simulated points to the nearest eradicated area were then calculated and compared with those obtained for the observed positives. This comparison allowed us to evaluate whether the progressively shorter distances observed over successive years could be explained by the expansion of eradicated areas. As these increased over time, the distance from any location within the study area to the nearest eradicated area naturally decreased. Additionally, distances between newly detected *Xf* positives and the boundaries of the infected area, defined by cumulative *Xf* positives from previous years, were calculated. Across all years, observed distances from newly detected *Xf* positives to eradicated areas were consistently smaller than those obtained from the simulations, indicating that new positives tended to occur close to areas where *Xf* had previously been identified. New *Xf* positives were most frequently found near the boundaries of the infected area. When positives were detected at greater distances, they occurred in areas with little or no prior surveillance and, therefore, cannot be univocally attributed to the spread of *Xf*. Overall, the observed limited spread of *Xf* in the outbreak in Alicante suggests that control measures are restricting pathogen expansion.

6.4 Detection of *Xylella fastidiosa* in important Mediterranean plants during dormancy (Euphresco project XfDorm)

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The multiplication of *Xylella fastidiosa* (*Xf*) within a host plant depends on environmental conditions, bacterial strains, and the species or cultivars of the plant. For trade purposes, the analysis of plant for planting is required. Sampling should preferably be performed during the plants' active growth period to maximise the likelihood of detection (Hopkins, 1981). In temperate zones, this period generally extends from late spring to autumn. In infected deciduous trees, the bacteria are not detected in the new season's growth until mid-summer, when symptoms may also become visible (EPPO, 2023). For deciduous plants, analysis is often required in autumn and winter, during the dormant phase when bacterial concentrations are the lowest. Experiments conducted within EU projects (POnTE, XF-ACTORS) have shown that *Xf* can be detected in some deciduous plants, all year round, including during the asymptomatic phase and dormancy. Although its detection has been shown to be possible in some cases, test performance depends on the plant species, geographical location and strains. In this context, a Euphresco project launched in 2023 aimed to evaluate the methods used to detect *Xf* and identify its subspecies in dormant Mediterranean plants. The performance of molecular tests was evaluated using both spiked and naturally infected dormant plant samples. A Test Performance Study (TPS) was organized, involving a total of 14 laboratories from 10 countries (EU, USA, Australia and UK). The panels distributed included 10 samples of dormant woody twigs naturally infected with different subspecies and sequence types of *Xf* as well as healthy samples. The "Harper simplex" PCR method was validated for the detection of *Xf* while the "Hodgetts simplex" and "Dupas tetraplex" PCR methods were validated for subspecies identification in dormant plant samples. In addition, within this project, *Xf*-infected almond trees were monitored throughout the year including dormancy in the Balearic Islands (Spain) and in Apulia (Italy). Detection of *Xf* was compared between petioles and wood samples. Wood samples were highly suitable for diagnostic analysis, as they provide relatively stable diagnostic sensitivity through the season. In conclusion, this project validated reliable methods for the analysis of *Xf* throughout the year, particularly during the dormancy stage, which are essential and highly relevant for the trade of economically important plants such as grapevines and *Prunus* species.

6.5 Large scale validation of qPCR assay for simultaneous identification of *Xylella fastidiosa* subspecies in a wide range of plant species naturally infected

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The genetic characterization of *Xylella fastidiosa* (*Xf*) isolates in EU is a legislative requirement; the EU Regulation 2020/1201 lists host plant species/genera according to their susceptibility to the different subspecies, and outbreak areas are demarcated according to the subspecies of the reported bacterial isolates. A complex phytosanitary scenario emerged in Apulia region (southern Italy) in 2024, with the finding of isolates genetically distinct from those of the subsp. *pauca* causing the olive epidemic in the region. Isolates of subsp. *fastidiosa* (ST1), *multiplex* (ST26) were found in the *Xf*-free area of the region, with a small area where the infected zones for the three subspecies overlap. It was then necessary to implement a diagnostic workflow integrating the detection of the bacterium with the identification of the subspecies for each positive finding. Briefly, all the DNA samples yielding positive results with the official qPCR assay of Harper et al. (2010) processed by the 4 laboratories of the regional network, are transferred at the reference laboratory of CNR-IPSP and subjected to qPCR using the combination of primers/probe designed by Dupas et al. (2019) to target in single reaction the three subspecies mentioned above. Reaction conditions were slightly modified to improve amplification signals for the subsp. *pauca*. Between 2024–2025 approx. 1500 DNA samples from positive field samples were subjected to subspecies identification and unambiguously categorized. Subspecies fastidiosa was mainly detected in Prunus dulcis (219 plants) and Vitis spp. (125), with occasional findings in *Prunus avium*, *Polygala myrtifolia* and *Prunus domestica*. Similarly, subsp. multiplex was identified mainly in almond trees (622) with few records in cherry (6), plum (4) and *Spartium junceum* (1). Subspecies *pauca* was exclusively associated with *Olea europaea* (508), with rare detections in almond. Positive samples from the area where all three subspecies coexist did not show any mixed infections, including in almond susceptible to multiple subspecies. The assay was also successfully extended to positive insect samples. For the purpose of the current legislation, our results showed that subspecies assignment through qPCR assay is a robust alternative to MLST analysis for a rapid and large-scale characterization of the *Xf* positive findings. The research was carried out within the official monitoring program organized by the Regional Phytosanitary Service of Apulia.

6.6 *Xylella fastidiosa*: from eradication to containment, the Porto metropolitan area (Portugal) case study

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Since the 2019 detection of the priority pest bacterium *Xylella fastidiosa* in the Porto Metropolitan Area, the northern region of Portugal has faced a complex phytosanitary crisis affecting multiple plant species, including ornamental shrubs, wild vegetation, and agricultural hosts. This study examines a six-year (2019–2025) diagnostic dataset (n=15,820) covering the Porto Metropolitan Area, northern Portugal, to evaluate pathogen establishment and spread. The bacterium is transmitted by xylem-feeding insects, including *Philaenus spumarius* in Europe and other vector species worldwide (e.g., sharpshooters, Cicadellidae), and is also disseminated through the movement of infected plant material. The bacterium infects plants, while insects act exclusively as transmission vectors. Results identified 512 positives (3.24%), covering 40,460 hectares. Spatial analysis revealed a maximum observed separation of 30.55 km between positive detections. Although this distance cannot by itself distinguish among dispersal mechanisms, its scale is consistent with a scenario in which local vector-mediated spread is complemented by human-assisted movement of infected plant material. Notably, positivity rates were nearly identical between symptomatic (3.04%) and asymptomatic (3.29%) plants (odds ratio=0.92), indicating that symptom occurrence is not predictive of infection status and highlighting the limitations of visual surveillance approaches. The coexistence of two distinct subspecies of *Xylella fastidiosa*, namely subsp. *multiplex* (sequence type 7) and subsp. *fastidiosa* (sequence type 2), together with high prevalence in wild hosts such as *Ulex micranthus* (48.72%) and *Cytisus scoparius* (27.12%), suggests a complex introduction history involving distinct genetic lineages and is consistent with ecological adaptation and widespread persistence within the local landscape. These findings justify the recent regulatory transition from an eradication-based approach to a containment strategy (Regulation (EU) 2024/2507) as the most appropriate management strategy under the current epidemiological conditions. This study highlights the necessity for risk-based monitoring and adaptive management in European peri-urban environments where *Xylella fastidiosa* shows evidence of sustained persistence.

Keywords: phytopathology; quarantine pest; subsp. *fastidiosa*; subsp. *multiplex*

6.7 Ten years of *Xylella fastidiosa* in the Balearic islands: impacts on crops and lessons from a multi-subspecies epidemic

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Xylella fastidiosa was first detected in the Balearic Islands in 2016, representing the first official record of the pathogen in Spain. Since then, extensive surveillance and numerous research activities have been conducted to monitor the outbreaks and advance our understanding of the pathogen's epidemiology under Mediterranean conditions. After a decade of continuous monitoring, the Balearic Islands have emerged as one of the most complex epidemiological scenarios for *X. fastidiosa* in Europe, with several subspecies and sequence types affecting a wide range of cultivated and wild plant species across the archipelago. In 2024, a new outbreak of *X. fastidiosa* subsp. *pauca* ST53, the same sequence type responsible for the devastating olive epidemic in Apulia (Italy), was detected in Mallorca. Interestingly, this outbreak remains highly localized within a restricted geographic area with a radius of approximately 2 km. Within this focus, plants infected by the three subspecies present in the islands (*pauca*, *multiplex* and *fastidiosa*) coexist, and mixed infections have been detected in some hosts, creating a unique epidemiological context where different subspecies interact within the same landscape. Here, we provide an updated overview of the *X. fastidiosa* situation ten years after the first detection, including new insights into the distribution of subspecies and sequence types, host range, and epidemiological patterns identified during long-term surveillance. We will also discuss the impact and behavior of different *X. fastidiosa* genotypes in the main crops of the islands, particularly grapevine, almond and olive, with special attention to the recent ST53 outbreak. In addition, we will summarize the main findings from research projects carried out in the islands on pathogen diversity, host–pathogen interactions and vector ecology. Overall, these observations indicate that the Balearic Islands constitute a unique natural laboratory for investigating the long-term epidemiology and evolutionary dynamics of *X. fastidiosa* in multi-subspecies epidemic scenarios under Mediterranean conditions.

6.8a Rapid volatilomic screening for *Xylella fastidiosa*: towards portable tools for phytosanitary surveillance

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The rapid emergence and spread of *Xylella fastidiosa* (*Xf*) in Europe highlight the urgent need for innovative rapid tools to support early diagnosis and large-scale phytosanitary surveillance. Current diagnostic techniques rely mainly on laboratory-based molecular analyses, which provide highly sensitive results but are not well suited for rapid and extensive field monitoring. Innovative diagnostic approaches, capable of detecting early physiological or metabolic alterations induced by the infection before the onset of symptoms, could therefore strengthen plant health surveillance, allowing the timely implementation of containment measures. Volatile organic compounds (VOCs) are secondary metabolites, emitted by plants, related to their physiological state and involved in several signalling mechanisms with the environment. In this work, the potentiality of using the volatilomic profile emitted by the plants as a prompt method for *Xf* detection at the early stage of infection was explored. The proposed strategy combines the headspace-solid phase gas chromatography–mass spectrometry (GC–MS) with a customized MOX gas sensor array that captures the total volatilomic fingerprints of plant–pathogen interactions, rather than the individual compounds. To in-depth analyze the metabolites of the rhizosphere, soil samples of different textures were collected near the plant root collar from resistant and susceptible olive cultivars and subjected to the measurements of volatilomic fractions. Preliminary results demonstrate that infected and control plants have different volatilomic signatures suggesting that the presence of the bacterial cells in the vessels significantly alters the composition and complexity of the soil microbiota and the surrounding microbial ecosystem. The response dynamics of portable MOX sensor arrays represents a solvent-free non-destructive tool to monitor the presence of *Xf* directly in the field, distinguishing between healthy and infected plants, and allows the identification of metabolites produced during the host-pathogen interaction. Future studies will focus on defining the optimal strategies for device placement in rhizosphere soil and on associating the detected volatilomic signals to possible metabolic pathways involved in the host defence or pathogen virulence mechanisms, before visible symptoms appear.

6.8b Horizon Scanning as a as an integrated approach on monitoring *Xylella fastidiosa*

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How is it possible to receive fast reliable information on pests and their spread and development worldwide? Europe is confronted with an increasing number of pathogens threatening agriculture and biodiversity. Horizon Scanning offers an approach how to keep an eye on these emerging threats: using the EIOS intelligence tool, we identify and extract scientific articles, news articles and social media posts which could contain signals about new and regulated pests. This way it is possible to detect important changes regarding distribution, host plants, vectors and potential management approaches. Methodologies like PeMo Screening allow a rapid assessment of the pest importance. One of the central emerging threats of the last years was and is *Xylella fastidiosa*, just recently reappearing in Bitonto (Puglia). To this day, this pest has been monitored continuously by the Horizon Scanning Working Group within EFSA. Based on this example we will present the workflow of Horizon Scanning, the tools involved, how to use the existing infrastructure framework, and what were the benefits in this case.

Session 7- Monitoring and modelling *Xylella fastidiosa* epidemics 2

7.1 Modelling the effectiveness of *Xylella fastidiosa* monitoring and control across pathosystems and spatial scales

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We have built a spatially explicit epidemiological model to predict the spread of *Xylella fastidiosa* under different control and inspection regimes. The model accounts for the differences between host species and host distributions across a range of case study outbreaks. It scales to the landscape sizes required to inform policy makers while maintaining the accuracy of an individual based model at smaller distances. This allows us to more accurately represent the impact of the spatial control strategies mandated in the current legislation i.e. radial inspection and removal. We use distance-dependent dispersal kernels to model transmission rate between hosts and model and track each host progressing stochastically through epidemic compartments with different compartment transition rates, infectivity and detectability per pathosystem. We also include seasonal variations in spread rate and per host disease progression. The model was parameterised using data from Apulia and through information obtained via expert knowledge elicitation. We use our model to evaluate eradication and containment effectiveness across exemplar epidemics of olive and table grape (based on Apulia), almond (Alicante) and a multi-host epidemic across wine grape and almond (Mallorca).

7.2 Enhancing *Xylella fastidiosa* spread modelling and surveillance optimisation through remote sensing

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Understanding the spread of *Xylella fastidiosa* (*Xf*) is essential for designing effective surveillance and management strategies. Epidemiological models are valuable tools for predicting disease dynamics, but their performance strongly depends on reliable spatial data describing disease distribution. Such data typically rely on field surveys, which are constrained by limited budgets and the long asymptomatic period of *Xf* infections, introducing spatial and temporal biases. Remote sensing (RS) enables large-scale mapping of disease distribution and can detect previsual symptoms of *Xf* infection, providing data which may improve the parameterisation of epidemiological models. This study aims to refine the parameterisation of a *Xf* spread model in olive using RS data, compare it with parameterisations based on field survey data, and evaluate the effectiveness of different surveillance and disease management scenarios. A spatially explicit, stochastic, discrete-time epidemiological model for *Xf* spread in olive was developed, capable of integrating both RS and field survey data. Two extensive field campaigns were conducted in *Xf*-affected olive orchards in Puglia (Italy) in 2016 and 2017, covering approximately 200,000 individual trees. Visual inspections assessed *Xf* incidence and severity, with qPCR testing in at least 10% of plants, including asymptomatic individuals, alongside airborne campaigns acquiring high-resolution thermal and hyperspectral imagery in the visible–near-infrared region (400–850 nm). A machine learning approach was applied to RS data to classify olive trees and estimate the probability of *Xf* infection. Results indicate that RS-based parameterisation yielded parameter estimates consistent with field inspection while producing sharper likelihood surfaces and better-constrained posterior distributions, indicating greater discriminatory power for model inference. Simulations showed that eradication under EU intervention guidelines requires surveillance at 1–3 month intervals, a frequency only achievable cost-effectively through RS. These findings demonstrate that RS, by providing improved spatial and temporal disease distribution data, enhances model parameterisation and enables optimisation of surveillance programmes that maximise detection performance while minimising costs.

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7.3 Adapting surveillance design to outbreak characteristics: a dynamic framework

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Epidemiological surveillance of *Xylella fastidiosa* relies on survey designs aimed to achieve a high probability of detection when the prevalence of the pathogen exceeds a predefined threshold. The sample size is usually determined using a hypergeometric approximation, by fixing a design prevalence (e.g., 1%) above which detection must be ensured with a given probability. However, this standard approach implicitly assumes spatial homogeneity in the distribution of the pathogen and does not account for the spatial characteristics of the outbreak, which plays a key role in both detectability and control. The literature clearly shows that epidemic dynamics and feasibility of control strongly depend on the spatial structure of the outbreak, such that clusters of diseased plants are often easier to detect and control than spatially dispersed outbreaks. Ignoring this spatial distribution may therefore lead to suboptimal survey designs, either by overestimating the required sample size or by reducing the actual probability of detection. We propose to explicitly incorporate spatial structure of the outbreak into the survey design. The spatial distribution of the pathogen in the outbreak area is modelled using a spatial Gaussian process, whose range parameter characterizes the level of spatial correlation and, hence, the degree of aggregation. Rather than arbitrarily fixing the same design prevalence across all contexts, we define range-specific threshold design prevalences. These are calibrated so that the expected perimeters in which prevalence exceeds the defined threshold are equal across scenarios with different levels of spatial aggregation. This geometric calibration ensures that, for different degrees of spatial aggregation, the resulting survey designs exhibit comparable performance in terms of efficiency and operational control capacity. In other words, the sampling effort is adjusted not only to the average prevalence but also to its spatial characteristics. Simulation studies illustrate the impact of the range parameter on some efficiency criteria and demonstrate how explicitly accounting for spatial structure of the outbreak allows for more efficient survey designs. Finally, we outline a sequential sampling framework for rapid detection of *X. fastidiosa*, based on area-interaction point processes and a Bayesian approach, in which spatial information accumulated over time is dynamically incorporated to adapt the sampling strategy.

7.4 Twelve years of models of *Xylella fastidiosa* epidemics in Europe: lessons learnt

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Mathematical and computational models play an increasingly role in epidemiology of plant pathogens and plant health, supporting the understanding and the evaluation of phytosanitary measures and Integrated Pest Management (IPM) strategies. Since the first official detection of *Xylella fastidiosa* (*Xf*) in Europe in 2013, a growing number of mechanistic epidemiological models has been developed to investigate disease dynamics and support *Xf* management. These approaches include classical compartmental frameworks, such as Susceptible–Infected–Removed models and their extensions including explicitly representing vector population dynamics and transmission processes, and formulations incorporating additional host compartments. Spatially explicit approaches have also been proposed, including reaction–diffusion models, lattice-based frameworks, and individual-based models. More recently, novel eco-epidemiological modelling frameworks have been developed within the BeXyl project, improving the representation of the *Xf* pathosystem by integrating key biological processes influencing disease dynamics, including pathogen development within hosts, vector population dynamics, and the influence of environmental factors on epidemic process. Overall, the modelling experience accumulated over the last decade highlights the critical role of epidemiological models in elucidating the ecological mechanisms underlying *Xf* epidemics and in providing a quantitative basis for risk assessment at European and national level, thereby supporting policy decisions and management strategies. Nevertheless, important knowledge gaps remain. Most models developed in Europe have focused on olive quick decline syndrome, while comparatively fewer studies have addressed other important *Xf* pathosystems. Future developments should therefore broaden the scope of modelling efforts and improve the representation of key epidemiological processes, particularly by incorporating the effects of environmental drivers on disease progression, vector population dynamics, and transmission processes. A more comprehensive integration of these components will strengthen models' capacity to support risk assessment and management of *Xf* across broader spatial and temporal scales. As management strategies progressively shift from emergency containment measures toward long-term IPM, modelling will play an increasingly important role in guiding timely and spatially targeted interventions for the effective management of *Xf*.

7.5 Evolution and consequences of nine years of application of phytosanitary measures to control *Xylella fastidiosa* subsp. *multiplex* outbreak in Alicante

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Introduction: The *Xylella fastidiosa* subsp. *multiplex* outbreak detected in 2017 in the province of Alicante triggered the immediate implementation of an eradication program based on European phytosanitary legislation. After nine years of application, it is essential to assess the spatial evolution of the outbreak, the effectiveness of the implemented measures, and their agronomic and social implications. This evaluation is necessary to determine whether the eradication strategy should continue or whether a transition to alternative management approaches is warranted.

Methods: The analysis is based on a review of the annual evolution of the demarcated area between 2017 and 2026, the implementation of mandatory measures (removal of host vegetation, vector control, and movement restrictions), and the prioritization criteria established by the regional administration. Data include affected surface area, annual expansion rates, outcomes of the interventions, satellite images comparison and evolution, and observations regarding societal response to the eradication program. Additionally, conditions in plots where measures were implemented were compared with those in areas where no interventions took place.

Results: The demarcated area exceeded 165,000 ha in 2025. While the initial years showed annual increases of more than 20%, the rate of expansion progressively declined and stabilized around 5%. Although complete eradication of the pathogen has not been achieved, the measures have significantly slowed its spread, allowing time for research developments and helping protect areas still free from infection. The prioritization of interventions enabled the implementation of measures without abrupt increases in budgetary requirements. At the same time, public perception has shifted from strong initial opposition toward more reasoned positions, even becoming supportive in the most affected areas. This shift is associated with the evident decline of plantations where measures were not applied, many of which are now abandoned due to reduced production and progressive dieback.

Discussion and conclusions: The data suggest that the eradication strategy has contributed to reducing inoculum levels and slowing disease progression, facilitating the regeneration of affected areas through alternative crops. Although full eradication has not been achieved, the territory is currently in a more favourable position to benefit from future research-driven solutions.

7.6 Integrated surveillance and phytosanitary measures against *Xylella fastidiosa* subsp. *fastidiosa* ST1, experience of the Apulia region

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The detection of *Xylella fastidiosa* subsp. *fastidiosa* ST1 (*Xff*) in grapevine, almond, and cherry in Puglia (Southern Italy) highlighted the urgent need to safeguard propagation material and grapevine production. Integrated surveillance combined plant inspections, vector monitoring, and molecular diagnostics to enable early detection, delineate the outbreak, and guide targeted phytosanitary interventions. Vector monitoring for *X. fastidiosa* (*Xf*) has been conducted continuously since 2017. In 2023, 190 monitoring sites were surveyed, capturing 2.667 spittlebugs (*Philaenus spumarius*), which were taxonomically identified and molecularly analysed. thirteen spittlebugs from Triggiano (in Bari province) tested positive for *Xf*. These detections prompted reinforced surveillance in olive groves vineyards, almond orchards, and surrounding host plants. Although the survey targeted *Xf* subsp. *pauca*, analysis of 432 host plant, mostly olive trees, discovered six *Xf*-infected almond trees, subsequently identified as infected by *Xff*. In 2024, an extensive delimiting survey defined the demarcated area, with over 44.000 plant samples collected and *Xff* detected in 212 almond trees, 119 grapevines, 7 cherry trees, and one Polygala. In 2025, a monitoring survey of 5.128 plants identified 3 almond trees and 8 grapevines positive for *Xff*, while 2026 surveillance of 330 samples, detected 10 positives. Eradication measures in the demarcated area included the removal of infected plants and host plants within a 50 m buffer. As part of these eradication measures, more than 30 hectares of table grape vineyards were uprooted. In addition, a more stringent phytosanitary measure was adopted for almond trees, which were removed within a 400 m buffer due to their role as major pathogen reservoirs. Nursery surveillance, covering over 11,000 propagation plants, confirmed *Xff* absence, ensuring the phytosanitary safety of the Puglia nursery sector. Mandatory measures of vector control were supported by a monitoring program of nymphs and adult vectors. These measures were complemented by a compensation scheme for affected farmers and nurserymen. Targeted communication and stakeholder engagement activities including technical meetings, seminars supported compliance with eradication measures. These coordinated plant health strategies effectively limited *Xff* spread, protected grapevine production and propagation material, and contributed to safeguarding European viticulture.

7.7 A hyperspectral phenotyping platform for early detection of *Xylella fastidiosa* infection in woody hosts

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Xylella fastidiosa (*Xf*) detection is hindered by asymptomatic infections, long incubation periods and non-specific symptoms that facilitate pathogen spread. Early detection before symptom onset is therefore essential to improve disease management. Hyperspectral imaging offers an alternative to conventional diagnostics by detecting subtle physiological changes induced by infection. To explore this potential, a phenotyping platform was developed for non-destructive monitoring of plant responses to *Xf* under controlled environmental conditions. The platform integrates vertical scanning to acquire high-resolution hyperspectral images using a sensor, covering the visible and near-infrared (VNIR) region (400 to 1000 nm). Hyperspectral data were acquired from woody crops, including olive, almond and grapevine plants artificially inoculated with *Xf* strains of different subspecies. Plants were monitored at multiple time points after inoculation and *Xf* infection was confirmed by qPCR analysis of plant tissue. An automated hyperspectral image-processing pipeline was developed, including in-situ calibration, spectral smoothing, and image segmentation using an ensemble of supervised machine-learning algorithms (89–99% global accuracy), followed by median filtering to obtain pure vegetation spectral signatures. Seventy vegetation indices (VIs) were calculated, filtered using the variance inflation factor (VIF), and used in machine-learning models to classify *Xf*-infected and non-inoculated plants (healthy). Initial results indicate that the system captures reproducible spectral data across crops. Subtle spectral differences between inoculated and non-inoculated plants were detected, in blue and NIR regions, in asymptomatic but infected plants. Indices related to chlorophyll degradation (NPQI), xanthophylls (PRIn), carotenoids (CRI700) and structural traits (MCARI) showed significant differences between treatments prior to visible symptom expression. Machine learning classification models successfully discriminated infected from non-infected plants, achieving validation accuracies above 70% and Kappa values exceeding 0.8. Overall, the phenotyping platform provides a robust tool for high-throughput monitoring of plant responses to *Xf* across woody crops supporting early detection strategies, epidemiological surveillance, and resistance screening in breeding programmes.

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Session 8- Pierce's disease of grapevines

8.1 Pierce's disease of grapevines: climate, diversity, and novel risks

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Pierce's disease of grapevines (PD) has been known as a disease that may occur in regions with mild winters. We now have field, greenhouse, in vitro, and genomic evidence that PD-causing *Xylella fastidiosa* in California are locally adapted, and that pathogen genotype and host plant variety are important in determining pathogen overwinter survivorship. These themes will be explored in addition to other recent findings impacting PD risk assessments, namely PD occurrence in areas with cold climates and the loss of varietal resistance in high pressure areas.

8.2 Impact of simulated climate change conditions on *Xylella fastidiosa*–grapevine interactions

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Understanding *Xylella fastidiosa* (*Xf*) infection response under climate change conditions is essential to predict future disease dynamics and potential spread. Although several models have projected an increased establishment of *Xf*-associated diseases under climate change scenarios, experimental validation of these predictions remains limited. This study evaluated grapevine response to *X. fastidiosa* infection under two simulated climate scenarios. The strain IVIA5770 (*Xf* subsp. *fastidiosa*, ST1) was inoculated in three grapevine cultivars (Sauvignon Blanc, Syrah and Chardonnay) grown under two irrigation regimes: field soil capacity and moderate water deficit (50% field capacity). Plants were maintained under current environmental conditions (24°C/400 ppm CO2) and two climate change scenarios (27°C/550 ppm CO2 and 30°C/750 ppm CO2). Symptom development was monitored over time using a 0-5 disease severity scale. Bacterial colonization was assessed by qPCR assays in leaves at 2- and 4- months post-inoculation (mpi) and several stem sections at the end of the experiment. Disease symptoms first appeared at 3 mpi. Analyses of the standardized area under the disease progress curve (sAUDPC) revealed significant differences of environmental scenarios (temperature and CO2), irrigation regime and cultivar. Higher sAUDPC values occurred under both simulated climate change scenarios, at 50% field capacity and Chardonnay cultivar. Bacterial detection frequencies were higher across all sampling points and plant tissues, exceeding 80%. In petioles, sampling time significantly influenced both detection frequency and bacterial load. In stems, the sampled plant section was the only factor that significantly affected detection frequency and bacterial load, a higher bacterial load was obtained above and below the inoculation point compared to non-inoculated secondary branches. Moreover, bacterial isolation resulted successful in more than 90% inoculated plants, confirming systemic infection and fulfilling Koch's postulates. The higher disease development observed at elevated temperature and CO_2 conditions suggest that future climate conditions may increase the severity of *Xf*-infections. These results provide relevant information and experimental evidence for risk assessment and disease management strategies under future climate change.

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8.3 Pre-winter pathogen load and cold exposure impact overwinter recovery from Pierce's Disease of grapevine

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Grapevine overwinter recovery from Pierce’s Disease is a well-established phenomenon that is thought to limit PD incidence and persistence in cooler climates. It is currently the only known cure for the disease. Despite its importance to disease risk assessments as well as range predictions under future climate scenarios, very little is known about the cold conditions or mechanism responsible for recovery from infections. We conducted a series of experiments over three winters to compare recovery across Cabernet Sauvignon plants at three California locations. Plants were removed four times over the course of the winter to create a range of temperature treatments. We constructed temperature models using this data as well as previously published recovery trial data to better understand the cold conditions associated with recovery. Past work has also shown that grapevine varieties recover at different rates. We performed RNA-seq on winter-collected cane samples from four grapevine varieties to investigate how gene expression patterns differ in higher and lower recovering varieties. Lastly, to investigate the longer-term effects of recovery on PD ecology, we ran a field experiment examining the impact of recovery on future reinfection susceptibility. Grapevines that had recovered from previous infections were mildly (11%) less likely to be reinfected than never-infected plants ($p = 0.061$, type II ANOVA). Future work aims to exploit these findings to assess disease risk and develop potential mechanisms to cure plants from *X. fastidiosa* infection.

8.4 Insights and prospects from the Apulian outbreak of *Xylella fastidiosa* subsp. *fastidiosa* in table grapes

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The recent detection of *Xylella fastidiosa* subsp. *fastidiosa* sequence type 1 (*Xff* ST1), the etiological agent of Pierce's disease, near the main table grape district in Apulia, raised major concerns for grapevine industry. Although field investigations were limited due to the eradication measures in place, surveys in infected vineyards provided preliminary evidence on the outcomes of the infections, cultivar susceptibility and vector-mediated transmissibility. Visual inspections and diagnostic assays were carried out in three young vineyards of the table grapes Superior Seedless® Sugraone, ARRA Sugar Drop™ (Arra30) and Melanie™ for a total of 1333 vines singly tested. Moreover, 159 plants were inspected and sampled in a small old vineyard for wine consisting of two mixed cultivars. Diagnostic tests showed an incidence >40% in all vineyards, except for Arra30 (9.90%). The incidence of infected vines positively correlated with the occurrence of grape mortality, with most of the dead plants confirmed to harbor bacterial DNA by qPCR. Leaf scorching and shrivelled berries were the symptoms most associated with positive vines. An average high bacterial population was estimated in the infected vineyards. Even if limited to a few trials of acquisition carried out limitedly in autumn, when individuals of *Philaenus spumarius* were caged on infected vines, positive specimens reached almost 30%. To extend the assessment on major wine and table grape cultivars, greenhouse tests were conducted by artificial inoculation including 10 cultivars, one rootstock and an open-pollinated progeny of *Vitis arizonica*. Inoculated grapevine plants and the rootstock resulted highly susceptible, exhibiting extensive bacterial colonization and typical leaf scorch, starting from 9–10 weeks after inoculation, with a progressive leaf loss and a general dieback. Re-isolation of the bacterium from infected plants further confirmed successful systemic infection. By contrast, most of the *V. arizonica*-derived plants harbored lower bacterial populations and displayed either delayed symptom onset or remained symptomless. Overall, these preliminary findings offer the first experimental evidence of the impact of *Xff*ST1 on grapevine in Southern Italy and reveal cultivar-dependent host responses that may be relevant for future investigation on the mechanisms underlying the resistance in grape and for breeding programs.

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8.5 Hidden Reservoirs of *Xylella fastidiosa* subsp. *multiplex*: Prevalence in Wild Hosts Surrounding Grapevine Regions in the Eastern USA

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X. fastidiosa subsp. *multiplex* is native to the eastern U.S. and infects many tree and shrub species. While its impact on landscape trees and crops like peach and blueberry is well-established, our previous work also identified subsp. multiplex in symptomatic grapevines in Virginia. Subsequent inoculations confirmed its ability to cause Pierce's disease of grapevines revealing a burgeoning risk to the viticulture industry. The role of wild vegetation as a "hidden" reservoir that can sustain pathogen populations and facilitate spillover into orchards and vineyards, remains poorly understood. Identifying these reservoirs is critical for predicting disease emergence and refining biosecurity strategies. In this study we assessed the prevalence and genetic diversity of *X. fastidiosa*, with a focus on subsp. multiplex, across wild plant communities adjacent to vineyards and orchards in 6 states throughout the Eastern USA. A total of 126 samples (# of species tested) with putative disease symptoms indicative of *X. fastidiosa* infection were collected. Detection and characterization were performed using qPCR, metagenomic sequencing, and culturing followed by genome sequencing. Our results reveal an unexpectedly high prevalence of *X. fastidiosa* across the surveyed geographic range, with a 95% infection rate among collected samples as determined by qPCR. Notably, we identified poison ivy (Toxicodendron radicans) growing adjacent to a vineyard as a novel host for subsp. *multiplex* and also detected it in a wild grape (*Vitis* spp.) sample, demonstrating the presence of *multiplex* reservoirs in and around vineyard ecosystems. The high infection rate and the identification of new plant species as hosts suggest substantial inoculum pressure on crops and underscore the need for surveillance and integrated management of buffer zones to minimize spillovers. Insights into population diversity, geographic distribution, and hosts of subsp. multiplex in its native region also informs risk assessment of subsp. *multiplex* in Europe, where it is being detected at increasing rates.

8.6 Effective Cover Crop Management in Vineyards Reduces the Abundance of *Philaenus spumarius*, the Main European Vector of *Xylella fastidiosa*

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Philaenus spumarius (L.) (Linnaeus, 1758) is the main vector of *Xylella fastidiosa* (*Xf*) in Europe. Since nymphs develop on herbaceous vegetation, ground cover management has emerged as a promising strategy that not only reduces vector populations but also enhances biodiversity and soil health. Several plant species with different levels of attractiveness for vectors can function as trap and repellent plants, ultimately decreasing vector abundance and thus reducing the risk of *Xf* transmission. To evaluate the effect of cover crops on *P. spumarius* abundance under field conditions, experiments were conducted in three vineyards located in Areeiro (Pontevedra), Cenicientos (Madrid) and Felanitx (Mallorca) in Spain. The abundance of *P. spumarius* nymphs was evaluated during spring under three cover crop treatments: spontaneous vegetation, *Sinapis alba*, and *Anthriscus cerefolium*. Cover crop composition significantly affected the abundance of nymphs. In Felanitx and Areeiro, nymph densities were significantly lower on rows sown with *Sinapis alba* than on spontaneous vegetation, whereas rows sown with *A. cerefolium* showed intermediate values that were not significantly different from the other treatments. In *Cenicientos*, only spontaneous vegetation and *A. cerefolium* were established, and ground covers containing *A. cerefolium* supported significantly fewer nymphs than spontaneous vegetation. Besides P. spumarius, other confirmed or potential *Xf* vectors such as *Neophilaenus campestris*, *Lepyronia coleoptrata*, *Cicadella viridis* and *Aphrophora alni* were detected. These results show that strategic cover crop management can significantly reduce *P. spumarius* nymph densities under realistic agronomic conditions, highlighting its potential as a sustainable strategy for effective vector management.

8.7 Climate-driven risk assessment of *Xylella fastidiosa* diseases: mechanistic modelling across scales

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The global expansion of plant pathogens is increasingly mediated by climate variability, yet current risk assessments often lack the mechanistic depth required to capture non-linear responses to environmental shifts. Here, we present a mechanistic modelling framework to assess the establishment and epidemic potential of *Xylella fastidiosa*, with a focus on Pierce's disease in grapevines. The approach integrates temperature-dependent epidemiological processes, vector spatial distribution and high-resolution climatic data to identify climatic thresholds, invasion conditions, and spatial heterogeneity in disease risk. Using historical and projected climate datasets, the framework quantifies present and future risk across global and European wine-growing regions, highlighting how warming alters epidemic growth rates and expands suitable areas, while also revealing strong effects of microclimatic variability that are missed by coarse-resolution assessments. Complementary phylogenetic and historical analyses provide context for why epidemics have so far been avoided in Europe and why this situation may change. Overall, the work illustrates how mechanistic, temperature-driven models can support the mandate of the European Food Safety Authority by improving climate-informed plant health risk assessment, horizon scanning, and evidence-based decision-making under uncertainty.

8.8a A novel and rapid in vitro method for assessment of *Vitis vinifera* susceptibility to *Xylella fastidiosa* subsp. *fastidiosa*

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Grapevine production, particularly in Italy, is of major economic and cultural importance; however, it is increasingly threatened by emerging plant diseases. *Xylella fastidiosa* subsp. *fastidiosa* (*Xff*), the causal agent of Pierce's disease (PD), colonizes grapevine xylem vessels, disrupts water transport, and causes leaf scorch, wilting, and eventual vine decline. Recently, *Xff* ST1 was reported for the first time in Apulia, southern Italy, raising concerns about the potential spread of this pathogen in Mediterranean viticulture. In this study, a novel in vitro screening method was developed to rapidly assess the susceptibility of Vitis vinifera genotypes to *Xff*. This approach was based on shoot culture micropropagation technique to obtain uniform plant material suitable for controlled inoculation assays. Using this method, eight autochthonous varieties, identified by the code numbers C1–C8, were inoculated with *Xff* in order to monitor bacterial colonization, disease progression, and symptom development under in vitro conditions. Quantification of bacterial populations revealed significant differences among the tested varieties, with higher bacterial loads (Log10 CFU mL^{-1}) detected in three of the eight grapevine varieties. Differential responses in susceptibility were observed over the 60 days following inoculation. Two varieties showed the highest susceptibility, whereas two others exhibited markedly lower susceptibility. Symptom progression also varied substantially among the tested varieties throughout the experimental period. Overall, the proposed in vitro system represents a promising rapid screening tool for the early evaluation of grapevine susceptibility to *Xff*. This methodology may support preliminary studies on host–pathogen interactions and provide a useful foundation for subsequent greenhouse- and field-based assessments.

8.8b Introducing the project XYL-o-VINE: Ecology, ethology and transmission biology of *Xylella fastidiosa* by insect vectors in table grape vineyards

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The emergence of *Xylella fastidiosa* subsp. *fastidiosa* Sequence Type 1 (*Xff*-ST1) in table grape vineyards in Apulia, Southern Italy, poses a significant new threat to European viticulture. Unlike well-studied outbreaks in olives, the epidemiology of *Xff*-ST1 in table grape systems remains poorly understood. Preliminary evidence suggests a bimodal transmission pathway involving *Neophilaenus campestris* (almond-to-grapevine) and *Philaenus spumarius* (grape-to-grapevine), but current data are spatially and temporally limited.

Poster session

P1. Physiological and hydraulic responses of three olive cultivars with contrasting responses to Xylella fastidiosa infection

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Xylella fastidiosa (*Xf*) is known to cause significant physiological changes in susceptible host plants, leading to the development of wilting and desiccation phenomena. In this study, we investigate the physiological mechanisms underlying olive cultivar responses to *Xf* infection by comparing the physiological and hydraulic responses of three olive cultivars differing in susceptibility to *X*f: Arbequina (susceptible), Leccino and Lecciana (both resistant). Ten one-year-old plants per cultivar were used; half were inoculated with *Xf* (infected plants, INF), while the remaining served as healthy controls (CTR). Two years after *Xf* inoculation, gas exchange chlorophyll fluorescence parameters were measured in both INF and CTR plants. Plant hydraulic functionality was also assessed by measuring leaf hydraulic conductance (Kleaf) and by evaluating xylem parenchyma vitality using the passive xylem staining technique. INF-Lecciana plants showed lower photochemical efficiency of Photosystem II (ΦPSII) and stomatal conductance (gs) values compared to CTR plants, resulting in lower photosynthesis (A). By contrast, Leccino and Arbequina showed a slight and non-significant reduction in A, gs, and ΦPSII in INF compared to CTR plants. INF plants of Leccino and Lecciana showed a reduction in Kleaf compared to CTR. Observation of stem-stained cross sections allowed quantification of non-functional xylem areas caused by *Xf* and supported Kleaf results. We hypothesise that hydraulic modulation observed in these INF plants may reflect the formation of callose-like material, a defence mechanism aimed at limiting *Xf* spread within the xylem already described in *Xf* resistant cultivars. On the other hand, Kleaf did not vary in response to *Xf* infection in Arbequina, potentially facilitating *Xf* spread through the xylem and accelerating symptom development at later stages of infection. Overall, physiological data did not show a positive correspondence with the severity of the symptoms, given that, two years from *Xf* inoculation, Arbequina plants showed severe desiccation phenomena while limited defoliation was recorded on Leccino and Lecciana. Thus, our results highlight complex mechanisms governing infection response in olive cultivars and suggest hydraulic modulation as a protection mechanism peculiar to *Xf* resistance cultivars.

This work is part of the project REACH-XY (State Budget Law of Italy N. 234, 30/12/2021).

P2. A multidisciplinary approach to understanding the response of olive cultivars to *Xylella fastidiosa*

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To identify new olive germplasm tolerant or resistant to *Xylella fastidiosa* (*Xf*), exploratory surveys were conducted in infected areas of Apulia. Several olive accessions showing intermediate symptoms severity were analysed for genetic identity, bacterial load, and feeding frequency of the vector *Philaenus spumarius*. No clear relationship was found between bacterial load and symptom expression, as some plants exhibited severe symptoms despite very low bacterial titres, suggesting that disease development is not exclusively determined by pathogen concentration. Genetic characterization, performed using ten SSR markers, showed that the paucisymptomatic accessions corresponded either to the *Xf*-susceptible cultivar Cellina di Nardò or to cultivars whose response to the bacterium is still unknown. Adult *P. spumarius* insert their stylets into olive xylem, mainly through the annual wood ring, the leaf petiole, and the midrib. During stylet penetration, salivary secretions are released and gelify, sealing the cuticle–plant interface and likely minimizing embolism formation. As a result of vector feeding activity, salivary sheaths remain within plant tissues after the feeding period. To estimate feeding pressure, the number of salivary sheaths per twig was quantified by examining fully developed one-year-old twigs. A rapid method based on hand-slicing and slide-mounting olive tissues preserved in 75% (v/v) ethanol/water allowed clear visualization and counting of salivary sheaths directly in planta. This approach made it possible to assess vector activity rapidly and comparatively among olive accessions. Quantifying vector access to host plants provides an estimate of the potential number of pathogen transmission events and allows the identification of accessions that are preferred, less accepted, or avoided by the vector, thereby supporting the development of more robust and effective clonal selection strategies. Overall, the study shows that the outcome of the *Xylella* pathosystem depends on the complex interaction among host plant genetics, pathogen virulence, and vector behaviour. Their combined effects may explain the observed dissociation between symptom severity and bacterial load and should be considered when identifying truly resilient olive genotypes. Therefore, double screening for both reduced susceptibility to *Xf* and reduced vector attractiveness or acceptance may represent a key strategy for designing more resilient olive orchards in Apulia.

P3. Developing and selecting novel *Xylella fastidiosa*-resistant, productive, best-performing and eco-sustainable olive genotypes

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The devastating impact of *Xylella fastidiosa* subsp. *pauca* (*Xfp*) in Salento olive cultivation necessitates the identification of resistant genotypes with high agronomic performance. Within the GENFORAGRIS project, over 550 cloned seedlings derived from inter varietal crosses are being evaluated, after confirmation of their hybrid origin through genotyping and parentage analyses. Following repeated diagnostic tests for *Xfp* detection, 70 trees were pre-selected, consistently testing negative or exhibiting extremely low bacterial loads, sometimes lower than the resistant cv. Favolosa. These genotypes have undergone comprehensive physiological, bio-morphological, and agronomic characterization on traits considered crucial for the establishment of new intensive olive orchards in the infected area, such as productivity, tree architecture, eco-sustainability, and metabolic profile of fruits and oil. Phenotypic analysis identified at least 10 most promising genotypes, selected for propagation and subsequent multi-site comparative trials. These results represent a significant step toward developing new, *Xf*-resistant, sustainable and high performing cultivars for olive oil and table olives production in infected regions.

P4. Xylem anatomy characterization of old olive plants belonging to traditional cultivars and supposed tolerant to *X. fastidiosa* in Salento

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Climate change is impacting the xylem hydraulic conductance of several perennial plants due to the higher incidence of embolism and implosion induced by high vapor pressure deficit during summer. As a form of long-term active acclimation, to prevent damages induced by embolism and implosion several plants reduce the xylem vessel diameter and increase the xylem vessel secondary wall thickness. This behaviour could also help plants to mitigate the damages produced by *X. fastidiosa* to xylem conductance. A survey was carried out in 2023 in Salento, an area strongly affected by *X. fastidiosa* since 2008 in South-Eastern Italy, to characterize xylem anatomy of pauci-symptomatic olive trees among strongly Olive Quick Design Syndrome (OQDS) affected plants, then possibly tolerant to *X. fastidiosa*. Xylem vessels diameter and vessels density, vessel wall thickness and lignification degree were detected by microscopic analysis of two years twigs thin sections; vulnerability index to cavitation was also calculated from xylem vessel diameter and vessels density. The results showed significant differences among plants examined, giving useful information for the possible acclimation of different old traditional genotypes to the environment, changing due to climate change, of this OQDS affected area.

P5. Exploring olive xylem metabolome: potential insights into *Xylella fastidiosa*-olive interactions

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In recent years, the worsening phytosanitary impact of *Xylella fastidiosa* subsp. *pauca* on the olive sector in Apulia, has prompted the development of new strategies for early diagnosis, control, and management. Among them, metabolomics has emerged as an interesting tool to investigate plant-pathogen interactions, providing insights that may be related to the biochemical dynamics underlying mechanisms of resistance and susceptibility. This study presents a targeted metabolomic analysis of olive xylem tissues using LC-MS/MS technology. Field sampling was conducted at two infected sites in Lecce (Apulia) and one bacteria-free site in the Olive World Germplasm Bank of Mirto Crosia (Calabria), allowing a comparative analysis between infected (*Xf*+) and uninfected (*Xf*-) plants. Three cultivars were selected based on susceptibility levels: Leccino (tolerant), Cellina di Nardò and Ogliarola salentina (susceptible). Seasonal sampling over one year enabled the assessment of temporal metabolic dynamics. Multivariate analyses confirmed clear separation between *Xf*+ and *Xf*- samples, with OPLS-DA showing high predictive ability (Q2 > 0.5) and statistically significant permutation tests ($p < 0.001$). Unpaired t-tests with false discovery rate (FDR) correction, VIP scores, and S-plot analyses highlighted verbascoside, oleuropein, quinic acid, and luteolin derivatives as key discriminating metabolites. Verbascoside was the most prominent, consistently increasing in infected plants: ~5-fold in tolerant Leccino, >20-fold in highly susceptible Cellina di Nardò, and ~8-fold in Ogliarola salentina, suggesting that accumulation may be proportional to cultivar susceptibility, with more susceptible varieties showing stronger but less controlled responses. Conversely, oleuropein generally decreased in infected plants, particularly in Leccino, likely due to enzymatic conversion into aldehydic derivatives, with potential cytotoxic effects on the invading pathogen, reflecting an active defense mechanism. Despite the inherent limitations of field-based studies, the consistent metabolic patterns observed suggest an association with *Xylella fastidiosa* infection and support xylem-targeted metabolomics as a promising approach for identifying chemical biomarkers, with potential applications in diagnostic development and breeding strategies for olive cultivars. The research activities were performed under the Project "DIACOX" (DM n. 664531, 28/12/2022).

P6a. Changes in the leaf proteome profile of susceptible and resistant olive cultivars in response to *Xylella fastidiosa* infection

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Xylella fastidiosa (*Xf*) has emerged as a major phytosanitary threat for olives, with isolates of the subsp. *pauca* being the primary cause of the olive quick decline syndrome, a severe disease affecting this species in the region of Apulia (Italy). Despite a wide number of olive cultivars turn to be susceptible, few displays distinct molecular and phenotypic responses resembling resistance phenomena. In this study, we performed a comparative proteomic analysis to investigate protein-level responses associated with *Xf* infection in two cultivars, the susceptible Arbequina and the resistant FS17. To capture host responses under natural epidemiological conditions, plants were selected in a 7-year-old olive grove located in the infected area, with trees of both cultivars planted in alternate rows. For each cultivar, samples (leaves) were collected from at least three positive and three negative trees. A total of 5461 proteins were identified using Liquid Chromatography - Mass spectrometry (LC-MS/MS), with significant changes in protein expression level observed when comparing the two cultivars and, within each cultivar, between infected and non-infected tissues. A set of 1096 proteins was down-regulated in infected trees of both cultivars. These proteins are involved in basic cellular processes, such as ribosomal structure and biogenesis, protein synthesis, nucleosome assembly, chromatin organization, iron metabolism and cell wall / galacturonate-associated processes. The up-regulated protein sets suggest that the two cultivars react differently to *Xf* infection. In FS17, infection is associated with activation of primary carbon metabolism and increased abundance of proteins involved in redox homeostasis, detoxification, electron transport and chaperone-mediated protein folding. In contrast, the susceptible cultivar Arbequina shows a marked increase in the abundance of expansion-like EG45 domain-containing proteins, which have been proposed to be related to plant natriuretic peptide-like signalling components and may play roles in stress signalling and physiological regulation. Our results reveal a substantial reprogramming of the olive leaf proteome in response to *Xf* infection and provide new insights into molecular processes potentially associated with olive cultivar responses.

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P6b. Deciphering local and systemic transcriptomic responses to *Xylella fastidiosa* infection in olive

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Olive turned to be a highly susceptible host to *Xylella fastidiosa* subsp. *pauca* (*Xfp*), whose infections cause, in sensitive cultivars, the olive quick decline syndrome. Recent studies indicate that olive responses to *Xylella* involve complex structural, biochemical and molecular mechanisms. To investigate transcriptional responses in field-grown trees, we selected infected and healthy trees of the susceptible cultivar Arbequina and the resistant cultivar FS17 grown in the same olive grove located in the demarcated infected area of Lecce (Apulia, Italy) thus exposed to natural infection of *Xfp* ST53. RNA-seq data, obtained by processing xylem tissues from 1-2 year-old shoots, were used to characterize the host transcriptomic response and to estimate bacterial colonization levels in the same tissue. Through a two-step mapping strategy, reads were first aligned to the olive genome and the remaining unmapped reads mapped to the genome of *Xfp* ST53 strain "De Donno" allowing to gather the relative bacterial abundance, a quality control step of the qPCR results yielded in each sample. A strong negative correlation between qPCR Cq values and RNA-seq-derived *Xylella* read confirmed the reliability of this approach, although the strength of the relationship differed between cultivars: $\rho = -0.96$ (Arbequina) vs $\rho = -0.59$ (FS17). A total of 26 total RNA libraries were sequenced, enhancing the investigation of two complementary biological effects: (i) differences between infected and healthy trees across cultivars, and (ii) local responses by comparing, in the same tree, branches with detectable and non-detectable bacterial populations. Cultivar-specific transcriptional responses were recorded, with several genes responding to infection only in Arbequina, whereas others were specifically modulated in FS17. Correlation analyses between transcript abundance and bacterial colonization identified candidate biomarkers associated with pathogen proliferation. In Arbequina, intra-plant comparisons highlighted a strong local transcriptional response to bacterial colonization, including induction of genes encoding EG45 domain-containing proteins and thaumatin-like proteins. Overall, these results show that olive transcriptional responses to *Xylella* are strongly cultivar-dependent and involve distinct local and systemic regulatory mechanisms.

This work is part of the projects OMIBREED (MASAF - decree n. 664766, 29/12/2022) and REACH-XY (State Budget Law of Italy N. 234, 30/12/2021).

P7. Genomic identification of loci associated with *Xylella fastidiosa* response in olive

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Xylella fastidiosa is a quarantine bacterium responsible for severe diseases in several plant species and the causal agent of Olive Quick Decline Syndrome (OQDS), which has severely impacted olive cultivation in Southern Italy. Understanding the genetic basis of resistance or tolerance to this pathogen is essential for developing sustainable mitigation strategies and supporting the recovery of affected olive-growing areas. Within the RIGENERA project, an integrated genomic and phenotypic approach has been implemented to identify genomic regions associated with olive response to *X. fastidiosa*. The study involves a panel of 200 olive genotypes, including 114 distinct Olea europaea cultivars and additional accessions from germplasm collections maintained at CREA-OFA (Rende, Italy) and the University of Bari. Whole-genome resequencing was carried out to characterize genetic variability and identify molecular markers associated with pathogen response. Variant calling analysis identified a genome-wide panel of single nucleotide polymorphisms (SNPs) using the genome assembly of the cultivar 'Leccino' as reference. To efficiently manage large genomic datasets, a bioinformatic workflow based on a NUMA-aware scatter–gather strategy was implemented, enabling parallel processing of genome intervals and improving computational efficiency during variant detection. In parallel, plant material from all selected accessions was propagated by rooting cuttings and grown under controlled conditions. Artificial inoculation with *X. fastidiosa* was performed on 12-month-old plants, while mock-inoculated plants served as controls. Disease progression will be assessed through visual symptom evaluation and quantification of bacterial load by qPCR at 6 and 12 months after inoculation. Genomic variants will be integrated with phenotypic data to build datasets suitable for genome-wide association studies (GWAS) aimed at identifying loci significantly associated with traits related to pathogen response. Candidate SNPs located within or near annotated genes will be prioritized to identify genomic regions potentially involved in resistance or tolerance mechanisms. The results will support marker-assisted selection and breeding strategies for the development of olive cultivars with improved resilience to *X. fastidiosa*.

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P.8 Genomic analysis of resistant cultivars to *Xylella fastidiosa* and their closest relatives

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The NOVIXGEN and RIGENERA projects (Italian Ministry of Agriculture, Food Sovereignty and Forestry) aim to support sustainability of olive cultivation by identifying tolerant or resistant genotypes against *Xylella fastidiosa*. This work focuses on genome resequencing of resistant cultivars and their closest relatives in order to reconstruct their genealogical relationships, as a starting point to shed light on the genetic basis of resistance. Capitalizing on previous studies of genetic relatedness, 26 cultivars were selected for short-read whole-genome resequencing, including Favolosa, Bianchella, Rosciola Laziale, Dritta di Loreto, Maurino, Pendolino, Zimbimbo, Colombina, Carboncella, Don Carlo, Donna Francesca, Sant'Agostino, Toscanina, Dolce di Cassano, Maiatica in NOVIXGEN, and Leccio del Corno, Cellina di Nardò, Ogliarola Salentina, Mignola, Oliva Rossa, Arbosana, Termite di Bitetto, Nolca, Fasolona, Giogolino, Giarraffa in RIGENERA. Raw reads from another 4 cultivars (Leccino, Frantoio, Grappolo and Coratina) were obtained from other studies or from databases. SNP variation was used for haplotype sharing analysis in order to reconstruct a family tree and investigate the association between haplotypes and the reported resistance or susceptibility to *Xylella*. All 4 cultivars that are authorized in Southern Italy for replanting orchards in *Xylella*-infected areas (Leccino, Favolosa, Leccio del Corno and Lecciana) descended from Frantoio and they are one or two generations away from their common ancestor. Despite this, Frantoio is not a resistant cultivar. Our analysis suggests that Leccino and Leccio del Corno are full-sibs and they have a parent-offspring relation with Frantoio, as well as they are half-sibs with Favolosa, sharing Frantoio as a common parent. This would imply that multiple unknown carriers of resistance, i.e. the missing second parents of these cultivars, are still unidentified, if resistance came from that lineage. We also identified other full-sibs of Leccino and Leccio del Corno as well as of Favolosa, which provide other potential resistant cultivars without prior knowledge of the genetic determinism. Frantoio itself may contribute beneficial alleles in a recessive or an additive model of resistance, expanding the set of potentially interesting cultivars to other relatives of Leccino, Leccio del Corno and Favolosa that are in a parent-offspring relationship with Frantoio.

P9. Evaluation of olive genotype response to olive quick decline syndrome caused by *Xylella fastidiosa* subsp. *pauca*

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Olive quick decline syndrome (OQDS), caused by *Xylella fastidiosa* subsp. *pauca* (*Xfp*), is one of the most destructive diseases currently affecting Mediterranean olive growing. Since its first detection in Apulia, southern Italy, in 2013, the pathogen has devastated extensive olive-growing areas, making the identification of resistant or tolerant genotypes a priority for sustainable disease management. In the absence of effective curative treatments, the use of resilient plant material remains the most promising long-term strategy. To identify potentially tolerant genotypes, extensive surveys were carried out in infected areas of Apulia over a six-year period, monitoring several putatively resilient olive genotypes, including centuries-old trees, through symptom severity assessment and bacterial quantification by real-time PCR. In parallel, five previously uncharacterized centenarian or millenarian olive trees have recently been identified in a heavily affected area where surrounding plants had died from the disease. These exceptional trees, described by the grower as highly and consistently productive, without marked alternate bearing, displayed morphological traits resembling the local cultivar Ogliarola Salentina and were included in the study as candidate resilient genotypes. A panel of SSR markers was used to assess the genetic relationships between the selected trees and widely cultivated Mediterranean olive varieties, enabling synonym identification through LRM analysis and phylogenetic clustering by the Unweighted Neighbor-Joining method. Paternity tests were also performed for previously unknown genotypes. The integration of molecular, phytopathological, and morphological data revealed marked variability in bacterial load and symptom expression, supporting the existence of different levels of susceptibility and resilience. Several genotypes showed close genetic relationships with resistant cultivars such as ‘Leccino’ and ‘FS17’, whereas others were related to local Apulian germplasm or to foreign cultivars such as the Albanian ‘Kalinjot’ and the Greek ‘Leucocarpa’. These findings highlight the strategic value of ancient olive germplasm as a reservoir of potentially resilient material for breeding, conservation, and landscape restoration in *Xfp*-affected areas.

P10. Olive responses to drought and *Xylella fastidiosa* subsp. *pauca*: an integrated physiological, biochemical, and hyperspectral assessment

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Xylella fastidiosa subsp. *pauca* (*Xfp*) disrupts xylem function leading to symptoms that mimic drought stress, such as leaf scorch and twig desiccation. These effects often intensify under the typical summer drought conditions of Mediterranean areas. To address this interaction, the present study investigates the physiological, biochemical and hyperspectral responses of olive trees under the pressure of *Xfp* and drought, specifically comparing Arbequina (susceptible) and Leccino (resistant) cultivars. We examined how drought and *Xfp* act individually and in combination in the two cultivars, under four conditions: well-watered control, *Xfp* infection, drought, and drought+*Xfp*. Measurements included stomatal conductance (gs), stem water potential (Ψstem), biochemical markers (H_2O_2, MDA, total antioxidant capacity), leaf hyperspectral reflectance and qPCR for detecting bacterial colonization. Furthermore, a gene expression analysis was conducted to assess the plant response by monitoring the relative expression of stress responsive genes. The results showed that drought induced the fastest and clearest shifts in gs and Ψstem, whereas *Xfp* alone caused smaller, cultivar-dependent changes within the experimental period. This pattern aligned with the hyperspectral data detecting pre-symptomatic water-stress signals in the visible and short-wave infrared regions only in the drought and combined stress, supporting its use as a non-invasive early-detection tool. In contrast, biochemical assays proved less informative: H_2O_2 and total antioxidant capacity changes were subtle, and only MDA showed consistent differences, being higher in Arbequina independently from the treatment. qPCR indicated low systemic infection rates more than one year after inoculation, suggesting uneven and limited bacterial spread over time. Visible additive effects of drought+*Xfp* emerged only in Arbequina toward the end of the experiment, whereas Leccino remained physiologically stable and symptom-free, consistent with its resistant phenotype. Overall, the rapid manifestation of drought effects versus the more gradual impact of *Xfp*, support a scenario of slow pathogen colonization and a prolonged asymptomatic phase, and underscore the need for longer term monitoring to fully capture the dynamics of drought–pathogen interactions.

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P11. Genome-wide characterization of the WAK/WAKL gene family in olive and expression patterns during *Xylella fastidiosa* infection

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Wall-associated kinases (WAKs) and WAK-like proteins (WAKLs) constitute a distinct subfamily of receptor-like kinases (RLKs) implicated in plant growth and development, as well as in abiotic and biotic stress-related signalling. Infections by *Xylella fastidiosa*, a bacterium that exploits the pit membranes (PMs) to spread within xylem vessels and colonize the host leading to disease developments, suggest the need to explore the potential role of these receptors in host–pathogen dynamics. In this study, we present a comprehensive in silico characterization of the WAK/WAKL gene family in olive (*Olea europaea* L.). The analysis of domain architecture, gene structure, and phylogeny was combined with transcriptomic profiling of the susceptible olive cv. 'Cellina di Nardò', upon infection with three genetically distinct *X. fastidiosa* strains belonging to subsp. *sandyi*, *pauca*, and multiplex, each inducing a different host response. The genome-wide survey identified a large repertoire of OeWAK/WAKL genes distributed across the O. europaea subsp. europaea cv. 'Farga' reference genome. All candidate targets were systematically analysed through computational prediction tools and manual inspection to verify the presence of a conserved intracellular kinase core and extracellular components, including epidermal growth factor–like (EGF) and galacturonan-binding (GUB) domains, along with a signal peptide (SP) and a transmembrane (TM) region. Duplication events were examined using MCScanX, and Ka/Ks values indicated predominant purifying selection. Maximum-likelihood phylogenetic analysis resolved five clades, highlighting the diversification of this gene family in olive. RNA-Seq profiling suggested that the expression patterns of the OeWAK/OeWAKL gene family may differentiate healthy from chronically infected plants and appear to be associated with the level of infection induced by the three bacterial strains. Integrated analysis of structural features and expression data yielded a prioritized list of OeWAK/OeWAKL candidate genes potentially involved in *X. fastidiosa* infection and/or symptom expression. To our knowledge, this represents the first in-depth analysis of the WAK/WAKL gene family in olive and lays the groundwork for further investigations into the *Xylella*–olive interaction. This research was carried out in the framework of the project "Research actions for reducing the impact on agricultural and natural ecosystems of the harmful plant pathogen *Xylella fastidiosa* (REACH-XY)" funded under the State Budget Law of Italy N. 234, 30 December 2021 (CUP B93C22001920001).

P12. Evaluating the response of Greek olive cultivars to *Xylella fastidiosa* subsp. *pauca* infection

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This study evaluates the susceptibility of three major Greek olive cultivars, Adramitini (AD), Throubolia Aegean (TR) and Kolovi (KO) to *Xylella fastidiosa* subsp. *pauca* (*Xfp*) under controlled greenhouse conditions. The primary objective was to determine whether these cultivars exhibit resilience comparable to the susceptibility similar to the highly vulnerable *Ogliarola Salentina* (OGS) or resistant Leccino (LC) cvs. Plants were inoculated in January 2023 and monitored by quantitative PCR (qPCR) to track bacterial colonization and symptoms in December 2024.

Results revealed a diverse range of responses among the Greek germplasms. While OGS is confirmed as the most susceptible with a Disease Index (DI%) of 91.43% and 86.42 % of desiccated shoot (DS%), the Greek cultivars showed different behavior. AD demonstrated the highest level of tolerance, maintaining low symptom severity (14.00 DI%) and 9.23 DS%, despite harboring the highest and most consistent bacterial concentration (4.44 Log10 CFU/mL). Tendentially, TR and KO showed a lower average bacterial load compared to AD, but none of the Greek germplasm differ statistically from OGS (3.94 Log10 CFU/mL). To assess the impact of infection on plant gas exchange, the stomatal conductance (g_s) was measured. The results showed that LC has stable g_s values between healthy and infected plants, whereas OGS exhibited a strong decline. AD and TR also showed marked reductions in g_s, indicating substantial physiological stress despite contrasting symptom severity, while KO displayed minimal changes. These measurements provided an additional physiological layer confirming cultivar-specific responses to *Xfp*. Hyperspectral reflectance (350–2500 nm) measurements were taken in January 2025, to identify physiological stress markers. The hyperspectral analysis proved to be a robust tool for non-invasive monitoring. Resistant varieties like LC (used as resistant control) maintained stable spectral signatures. In contrast, infected Greek cultivars and OGS showed significant spectral deviations, specifically a collapse in the visible range (VIS) and Near-Infrared (NIR) plateau. In conclusion, the findings highlight that while these Greek varieties are susceptible to *Xfp*, their symptomatic progression seem to be generally less aggressive than that of OGS. The integration of spectroradiometry offers a high-throughput method for monitoring physiological decline, with significant potential for future spectral-based approaches.

P13. Characterization of an olive germplasm collection for response to *Xylella fastidiosa*

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Xylella fastidiosa (*Xf*) represents one of the most severe phytosanitary threats to Mediterranean agriculture, severely affecting olive cultivation in Apulia (Southern Italy), where the pathogen has spread rapidly since its introduction in 2013. The characterization of genetic variation response to *Xf* and the genetic basis of resistance is therefore crucial. Within the framework of the project Novixigen, we are currently performing the characterization of 100 olive cultivars maintained in the national facility of the Italian Council for Agricultural Research and Economics (CREA), located in Monteroni (Lecce), an area heavily affected by *Xf* infection. Specifically, genetic characterization is currently ongoing by whole-genome resequencing (WGS). In addition, the same phenotypic evaluation will be performed with respect to the level of bacterial colonization and other traits potentially correlated with *Xf* resistance. Genome-wide association studies (GWASs) will be carried out to identify genomic regions, molecular markers, and candidate genes potentially associated with phenotypic variation. Expected outcomes include the characterization of cultivars with enhanced resistance and insights on molecular mechanisms underlying the olive-*Xf* interaction.

P14. Short periods of high temperature and water deficit induce different responses in two olive cultivars tolerant to *Xylella fastidiosa* subsp. *pauca*

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Several short-term experiments on the effect of heat and drought stress were conducted in growth chambers using young, vegetative plants of two olive cultivars (FS 17, Leccino) tolerant to *Xylella fastidiosa* subsp. *pauca* (*Xfp*). Because of the dearth of information on the response of both cultivars to climate change, in a typical experiment we exposed container-grown plants to the combined effect of high temperature (max. 43 °C, min. 23 °C) and water deficit (either well irrigated or less irrigated) for 7-9 days under a 16/8 h day/night photoperiod. Temperature and photosynthetic photon flux were set to change stepwise between minimum and maximum values, relative humidity was set at 50%, CO2 partial pressure at 430 ⍰Pa Pa-1. Gas exchange parameters, chlorophyll a fluorescence, water potential, chlorophyll concentration, chromatic constants of fully expanded leaves were measured during the experiments. The two cultivars responded differently to the concomitant effect of heat and water stress. The cv. Leccino tended to increase gas exchange parameters after few days of heat exposure, probably in an attempt to compensate for the higher temperature by thermoregulation. However, when the water supply was limited (plants receiving less water than they actually transpired) gas exchange parameters dropped and extensive leaf damage and plant mortality appeared. The cv. FS 17 plants were more water conservative when exposed to high temperatures, their stomatal conductance and transpiration increased less than in the cv. Leccino and so consumed less water and maintained better water status. The differences between cultivars appeared only in plants receiving limited volumes of irrigation, whereas both cultivars showed similar behaviour when plants were fully irrigated or kept at normal temperature. These results are important to guide growers in varietal choice at planting and cultural practices during training of new olive orchards in the *Xfp*-infected area.

P15. Sensitivity of young olive plants of cultivar ‘Lecciana’ to heat and water deficit

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In 2025 an experiment was conducted on potted olive plants of cultivar ‘Lecciana’ under controlled environment conditions to assess the resistance of this new cultivar to the combined effect of water deficit and high temperature. ‘Lecciana’ is considered tolerant to *Xylella fastidiosa* subsp. *pauca* (*Xfp*) and it is, therefore, allowed to be planted in *Xfp*-affected areas of Apulia. The experiment consisted of subjecting 16 plants (either fully-irrigated or non-irrigated) to 7 days of standard (max. 23 °C – cool chamber) and high temperatures (max. 43 °C – hot chamber) under a 16/8 h day/night photoperiod in the two different growth chambers. Leaf gas exchange parameters, chlorophyll a fluorescence, water potential and chromatic constants of fully-expanded leaves were measured. Shoot apices and leaves showed evident signs of damage after 7 days of non-irrigated plants in the hot chamber, whereas fully-irrigated plants or those kept in the cool chamber did not show any symptom of damage. Significant reductions in gas exchange parameters due to water deficit started appearing 3 days after the beginning of the experiment under both temperature regimes, the Fv/Fm fluorescence decreased for non-watered plants in the hot chamber after 5 days. By the end of the experiment the leaf water potential of plants under high temperature had reached -6.03 and -2.98 MPa for droughted and fully-irrigated treatment, respectively, whereas it was about -5.09 and -1.14 MPa for respective treatments in the cool chamber. Our results show the relevance of irrigation during the training phase of 'Lecciana' to cope with heat waves and high temperatures.

P16. Exploring olive tree resilience through biodiversity: an "open air laboratory" in the infected area of salento to evaluate the resistance of olive cultivars to *Xylella fastidiosa* subsp. *pauca*

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A multivarietal olive experimental field was established in 2019 in Salento (Apulia Region; Collepasso, province of Lecce), in an area infected with *Xylella fastidiosa* subsp. *pauca* (*Xfp*), the causal agent of Olive Quick Decline Syndrome. The study evaluates olive cultivars (cvs.), native to Molise and neighbouring regions, in terms of resistance or susceptibility to natural *Xfp* infections, agronomic adaptability and productivity as well as incidence and severity of other endemic olive pathogens. The field, established according to a randomized block design, includes the susceptible local cvs. Ogliarola Salentina and Cellina di Nardò, together with the national resistant cvs. Leccino and Favolosa. Multi-year monitoring revealed marked differences among cultivars. The Campanian cv. Racioppella showed exceptional susceptibility, with severe symptoms, whereas the Molisan Olivastro di Montenero and the Tuscan cv. Leccio del Corno, remained symptom-free. Leccio del Corno exhibited resilience comparable to that of Leccino, supporting its recent authorization for planting in infected areas. The cv. Leccio del Corno also proved highly productivity, with early bearing and stable yields. The Molisan cv. Cornarella di Guglionesi displayed only mild symptoms. qPCR analyses confirmed a widespread *Xfp* presence in the experimental field, with significant variation in bacterial loads; cv. Olivastro di Montenero showed the lowest bacterial concentration and infection rate among all tested cultivars. A clone of the cv. Leccino exhibited enhanced resistance and reduced alternate bearing compared with the standard Leccino. The cvs. Frantoio and Cerasa di Montenero, which are also sensitive to *Xfp*, proved highly susceptible to Cercospora leaf spot and peacock leaf spot. Beyond its scientific value, this “open-air laboratory” promotes technology transfer. Strong farmer interest has fostered direct dialogue on innovations to address the *Xfp* crisis. This synergy is further enhanced by a plot planted in the same field with young olive trees susceptible to the pathogen, aimed at testing innovative and sustainable control strategies, accelerating the transition from research to field application.

P17. Exploring intravarietal diversity in Leccino olive cultivar for resistance to *Xylella fastidiosa*

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Over the last decade, the spread of *Xylella fastidiosa* subsp. *pauca* (strain ST53) in Apulia region has severely threatened a regional economy largely based on olive cultivation and olive oil production. To counteract the diffusion of the pathogen, several regional and national projects have focused on the evaluation of olive biodiversity to identify new sources of resistance and to better understand host response mechanisms. To date, the olive cultivars recognized as resistant to *Xylella fastidiosa* include Leccino, Favolosa, Lecciana, and Leccio del Corno. However, despite the overall resistant behavior of the Leccino cultivar, recent studies have highlighted the presence of intravarietal variability. Field surveys conducted in severely affected areas of Salento revealed occasional presence of Leccino trees that are characterized by some symptom expression, despite an overall attenuated phenotype. Real-time PCR assays carried out over four consecutive years (2023–2026) on 20 Leccino trees revealed fluctuations in bacterial load, suggesting the possible occurrence of clonal variation for the modulation of resistance. High-molecular-weight nuclear DNA from the sampled plants is currently being sequenced to investigate somatic mutations that might explain differential responses to *Xylella fastidiosa*. In parallel, chemical analyses are being performed to assess potential metabolic differences among clones. The integration of genomic and secondary metabolite data may provide valuable insights into the molecular determinants of resistance and contribute to the identification of genes involved in the olive response to the pathogen.

P18. Leccino clones: metabolomic preliminary insights into *Xylella fastidiosa* tolerance

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The NOVIXGEN Project (DM n. 0664890, 29/12/2022), whose interdisciplinary core allows, among other things, the investigation of *Xylella fastidiosa*-olive interactions at multiple levels, aims to better understand the mechanisms underlying tolerance and susceptibility among different olive cultivars. The present work focuses specifically on the cultivar Leccino, known for its relative tolerance to the pathogen. A metabolomic characterization was performed on 11 Leccino clones selected by the University of Bari 'Aldo Moro', collected in infected areas of Salento and exhibiting varying degrees of symptom severity. Leaf tissue analyses were conducted using LC-MS/MS across three distinct seasonal samplings. Simultaneously, high-molecular-weight genomic DNA is being extracted from each olive clone. Preliminary evaluations (ANOVA and PLS-DA) revealed a significant seasonal influence on metabolome and key discriminant metabolites confirmed a seasonal shift between primary and secondary metabolism. To isolate clone-specific differences, multivariate analyses were conducted separately for each season. Results indicate that, although seasonality strongly affects the overall metabolomic profile, clones also display distinct metabolic patterns, with some maintaining relatively stable profiles across seasons and others exhibiting higher variability. To identify metabolites contributing most to clone discrimination, VIP scores were evaluated, and hierarchical cluster analysis used to assess similarities among clone profiles. Combined interpretation revealed persistent metabolomic clusters, with some clones consistently showing higher levels of defense-related metabolites such as oleuropein, hydroxytyrosol, and maslinic acid, while others maintained lower or intermediate profiles across all seasons. These preliminary data, integrating metabolomics and DNA sequencing, provide a framework to identify metabolic pathways and candidate molecular biomarkers associated with tolerance. This clarifies how different clones translate genetic signals into specific chemical barriers capable of limiting pathogen spread within xylem tissues, elucidating the molecular mechanisms underlying the plant’s response to the bacterium.

P19. Development of effective embryogenic callus induction and regeneration protocols addressed to specific olive cultivars

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Developing effective protocols for olive (*Olea europaea* L.) embryogenic callus induction from adult explants is a crucial, yet underdeveloped, step to unlock the potential of the New Genomic Techniques (NGTs) application in the genetic improvement of this important crop. To date, only a few protocols starting from mature explants are available, and even fewer have been developed for more recalcitrant varieties. The aim of the present study is to obtain embryogenic callus as starting material for genome editing approaches, with the long-term goal of conferring increased tolerance to varieties susceptible to the pathogen *Xylella fastidiosa*. In this regard, different explant types and culture media were tested on three olive varieties with a different degree of susceptibility to the pathogen: the tolerant 'Favolosa' and the susceptible 'Kalamata' and 'Arbequina'. The accessions showed variable responses to the callus induction media depending on the explant characteristics. Different salts, sugar concentrations, and the presence of other compounds, such as silver nitrate (AgNO3), have been observed to alter the structure and morphology of the calli tissues, with some protocols promoting higher numbers of pre-embryogenic growths. At the same time, various hormones combinations and concentrations have shown very different responses between the three genotypes. To date, recent modifications in the protocols are producing promising results, such as 'Favolosa' exhibiting, on average, a higher frequency of embryogenic-like structures, and 'Kalamata' developing the first shoots. These preliminary findings indicate that the induced callus exhibits both embryogenic and regenerative competence; however, a cultivar-specific protocol is required and is currently under development for different commercial cultivars.

P20. Genetic mapping of resistance loci to *Xylella fastidiosa* in olive

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The economic stability, landscape, and agricultural heritage of the Apulia region in Southern Italy have been severely affected by the bacterium *Xylella fastidiosa* subsp. *pauca* (*X.f.*) since 2013. Transmitted by the spittlebug *Philaenus spumarius*, *X.f.* colonizes the xylem vessels of olive trees, eventually leading to their decline. In Europe, annual surveillance programs for *X.f.* support phytosanitary measures aimed at limiting its spread following the outbreaks in olive trees in southern Italy. These programs involve inspection and sampling of host plants, particularly symptomatic or susceptible species, and have revealed multiple outbreaks and a wide host range across several countries. However, the mechanisms underlying resistance to *X.f.* remain poorly understood. Biparental linkage analysis, utilizing high-throughput genotyping as genotyping-by sequencing (GBS), offers a powerful method for improving the precision and efficiency of Quantitative Trait Loci (QTL) mapping. This approach is designed to unravel the genetic architecture of complex traits, specifically resistance to *X.f.* in olives. This research is part of the RIGENERA project (Approcci IntegRati per il mIglioramento GENEtico, la selezione e l'ottenimento di materiali vegetali Resistenti a *Xylella fastidiosa*), which aims to explore the genetic basis of resistance and susceptibility in olive trees through a multidisciplinary approach. The segregating population analyzed in this study was generated by crossing the *X.f.*-resistant cultivars Leccino and FS17, maintained at the Basile Caramia Research Center (CRSFA) in Locorotondo (Bari). The population comprises 261 F1 genotypes. To ensure the purity of the progeny, the parents and offspring were screened using two highly polymorphic microsatellite markers (DCA03 and DCA17) to identify and exclude individuals resulting from selfing or accidental cross-pollination. Of the 261 initial genotypes, 183 were confirmed as true Leccino x FS17 offspring, while 34 originated from Leccino self-pollination and 44 from crosses with other olive trees. The verified F1 plants are currently undergoing propagation and experimental infection. Simultaneously, these lines are being analyzed via GBS to develop a customized Single Nucleotide Polymorphism panel for linkage mapping. Integrating genetic linkage data with the phenotypic responses of F1 plants to *X.f.* will be essential for identifying the genomic regions and candidate genes associated with olive resistance.

P21. A target gene panel for screening of tolerant/resistant olive genotypes to *Xylella fastidiosa*

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An extremely important objective that can be pursued by expanding ongoing research is the intensification of varietal screening, extending it to as many olive tree accessions as possible. At the same time, actions aimed at safeguard cultivars at risk of extinction, currently confined to very limited areas, could also be undertaken. To develop a target gene panel for screening of olive genotypes tolerant or resistant to *Xylella fastidiosa*, a search for candidate genes was performed through a meta-analysis of publicly availably RNA-seq libraries. The sequences were processed using RNA-seq pipeline including alignment to the 'Leccino' reference genome, gene expression quantification and differential expression analysis to identify genes regulated in response to the infection. Functional enrichment analysis (Gene Ontology, GO) was then performed to characterize the biological processes (BP), molecular functions (MF), and cellular components (CC) associated with the differential response of the cultivars. In particular, several BP-associated genes involved in responses to biotic and abiotic stresses, especially drought stress, were found to be upregulated in *Xylella*-tolerant cultivars; modifications in membranes and cell walls of xylem vessels could favor a pathogen compartmentalization, likely creating a physical barrier that limits its spread. On the other hand, BPs related to responses to other organisms and to the regulation of stimulus responses were found to be upregulated in cultivars susceptible to the bacterium. The availability of the reference genome assembly of the cultivar Leccino, for which both haplotypes are accessible, allowed the identification of alternative forms of the same target genes. The ultimate goal is to develop a panel of genes potentially involved in the response to *Xylella*, to be used in a combined and scalable manner. This panel could represent a rapid, cost-effective, and easily accessible tool for the scientific community to phenotype olive genotypes and support varietal selection based on their different levels of tolerance to *Xylella fastidiosa*.

P22. Identification and genetic characterisation of olive genotypes potentially tolerant/resistant to *Xylella fastidiosa* in the Apulia region

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X. fastidiosa subsp. *pauca* was first identified in 2013 in Apulia (near Gallipoli) and rapidly spread across the region. This quarantine pathogen colonizes xylem vessels and is transmitted by insect vectors such as *Philaenus s.* Once inside the host, it proliferates in the xylem, blocking vessels via bacterial aggregates and host-produced tyloses and gums, with symptoms often resembling water stress. In olive trees, symptoms include desiccation of twigs and small branches, initially in the upper canopy and progressively spreading to the entire crown, eventually causing decline and death in susceptible cultivars within a few years. The rapid spread was favored by the lack of effective control strategies and the predominance in Salento of the local cv C. di Nardò and *Ogliarola s.*, both highly susceptible, leading to extensive tree mortality and major economic and production losses. Increasing olive biodiversity represents a key strategy for recovery and long-term sustainability of affected areas. In this study, as part of the Novixgen project, olive genotypes potentially tolerant or resistant to the pathogen were identified and analyzed. An extensive field survey in the initially affected Salento areas, integrating GIS and field observations, selected 200 olive trees showing no or mild symptoms indicative of potential tolerance. These plants were genetically characterized using SSR markers, and their profiles were compared with a dataset of national and international olive cultivars. Preliminary results revealed that many genotypes did not match known varieties, suggesting spontaneous crosses or local accessions not attributable to widely characterized cultivars. Paternity analyses revealed that, in some cases, a relationship with the Leccino cultivar, known for its tolerance to *Xf*, was detected, as well as close genetic relationships with cultivars whose response to the pathogen is still unknown. These results highlight the need for further investigation of genotypes not yet characterized for resistance or tolerance to the bacterium. Phylogenetic analyses were conducted to clarify relationships between selected genotypes and reference cultivars. Identification and genetic characterization of tolerant genotypes provide valuable information for breeding programs and the selection of resilient nursery material, supporting the restoration of olive cultivation in Apulia and enhancing the resilience of Mediterranean olive agroecosystems affected by the pathogen.

P23. Callus elicitation as a tool to identify key genetic responses to *Xylella fastidiosa* in olive (*Olea europaea L.*)

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The severe impact of the Gram-negative bacterium *Xylella fastidiosa* subsp. *pauca* (*Xfp*) on olive cultivation in Apulia region has highlighted the need for deeper insight into plant defence mechanisms and the genes involved in resistance. To develop a rapid screening approach for varietal resistance or susceptibility, leaf and petiole explants from the susceptible cultivar Ogliarola salentina and the resistant Leccio del Corno were cultured on NN (Nitsch and Nitsch, 1969), MS (Murashige and Skoog, 1962), and CIM (Callus Induction Medium) culture media with modified hormonal compositions, resulting in genotype-dependent callus production. Calli derived from Ogliarola salentina were subsequently elicited with a *Xylella fastidiosa* lysate (*Xf*L) at different time points and concentrations and analysed for defence-related secondary metabolites using LC–MS/MS. Metabolomic profiling revealed the accumulation of polyphenols, including hydroxytyrosol, oleuropein, and verbascoside, as well as terpenes such as oleanolic, maslinic, and corosolic acids. Notably, elicitation induced a marked accumulation of oleuropein, a key olive secoiridoid involved in defence responses to biotic stress. To validate the regulatory effect of the bacterial lysate on gene expression and to support the metabolic pathways identified by chemical analysis, transcript levels of the genes OePAR, OeBAS, OeTYRD, OeCuAO, OeADLH, OeCYP716C67, OePAL and OeC4H were quantified by real-time PCR, highlighting significant differences in expression at different time points and *Xf*L-doses. Overall, the study aims to identify genes involved in polyphenol and terpene biosynthesis in order to clarify their role in olive defence responses, particularly in relation to *Xylella fastidiosa* infection.

P24. Exploring olive microbiome composition in relation to rootstocks and *Xylella fastidiosa* subsp. *pauca* infection

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Xylella fastidiosa subsp. *pauca* is the causal agent of Olive Quick Decline Syndrome (OQDS), a serious threat to olive production across the Mediterranean basin. Understanding the interactions between the pathogen, host plants, and associated microbial communities is essential for improving disease management and identifying potential resilience factors in olive cultivars. Among these factors, the influence of rootstocks on plant-associated microbiomes may play an important role in modulating plant susceptibility or tolerance to infection. Sampling activities took place in March 2025 in a semi-intensive olive orchard located in the province of Brindisi (Apulia, Italy). The field included nine different self-rooted olive cultivars, four of which were also grafted onto three different rootstocks. For each cultivar or rootstock combination, ten plants were selected for the collection of leaf and root samples. DNA was extracted from leaf and root samples in order to prepare sequencing libraries for 16S and ITS2 amplicon sequencing. Through bioinformatics analyses, we identified olive trees infected by *X. fastidiosa*, providing information on the distribution of the pathogen among the different cultivars and rootstock combinations present in the field. We also characterized the bacterial and fungal communities associated with infected and uninfected plants. Comparing microbial profiles between different cultivars, rootstocks, and plant health conditions may reveal patterns of microbial association potentially linked to plant susceptibility or resilience to infection. Overall, this study will contribute to a better understanding of the complex interactions among *X. fastidiosa*, olive plants, and their microbiota. The results may provide useful information for future strategies aimed at mitigating the impact of OQDS and supporting sustainable olive cultivation in affected regions.

P25. Genome editing in olive: from embryogenic callus induction to a protoplast-based platform

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P26. Germplasm management of an olive core collection to investigate genetic resistance to *Xylella fastidiosa* subsp. *pauca*

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Within the framework of the Rigenera project, a national facility was established to conserve and characterize olive genotypes in relation to their response to infection by *Xylella fastidiosa* subsp. *pauca* (strain ST53). A core collection of 100 olive genotypes, selected on the basis of high genetic distance, was defined according to the results of GBS (Genotyping-by-Sequencing) and SPET (Single Primer Enrichment Technology) analyses performed on a broader germplasm collection maintained at CREA-OFA. These genotypes were propagated to establish a transcriptomic trial with different replicates in order to evaluate their response to bacterial infection. During varietal multiplication, 34 genotypes, including the resistant cultivars Leccino and Leccio del Corno and the tolerant cultivar Frantoio, showed high propagation aptitude. By contrast, 18 genotypes exhibited low propagation ability, while 48 genotypes, including Cellina di Nardò and other minor varieties, confirmed the recalcitrance of olive to propagation, thus requiring grafting. The 34 genotypes with high propagation aptitude were transferred to a controlled greenhouse environment and artificially inoculated in order to perform transcriptomic analyses. Inoculation procedures were carried out twice, as only a limited number of genotypes showed a significant bacterial load during the first infection cycle. The same experimental protocol will be applied to the remaining genotypes as soon as suitable plant material becomes available. Overall, appropriate germplasm management proved to be a critical factor for the successful implementation of the experimental activities and for the achievement of the project objectives, highlighting its central role in resistance studies and in the functional genomics of olive–*Xylella* interactions.

P27. Soil microbial diversity in fields with olive trees tolerant and non-tolerant to *Xylella fastidiosa*

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Xylella fastidiosa (*Xf*) (subsp. *pauca*) infection was established more than a decade ago in Italy, causing widespread emergence of the Olive Quick Decline Syndrome (OQDS). Its progression has led to severe landscape and biodiversity losses, as well as substantial economic damage to the olive sector. A growing interest lies in identifying tolerant genotypes of autochthonous cultivars both to preserve product typicity and to elucidate the mechanisms underlying tolerance. Within this framework, the NOVIXGEN project aims to identify, monitor, and characterize olive genetic material with potential resistance or tolerance traits to *Xf* for propagation. Plants, like other organisms, form holobionts with their associated microbiota rather than isolated genomic entities. Indeed, the microbial community that inhabits the soil, particularly that associated to the root system, is known for positive interactions with the plant modulating its health and responses to abiotic and biotic stress, for example through the stimulation of systemic acquired resistance. Accordingly, this study investigated whether the soil microbiome may contribute to tolerance mechanisms in different olive cultivars. The study area was located in the Apulia region where *Xf* infection was initially established in 2013 in the southern Salento (Gallipoli-Otranto zone). Here, three fields were selected based on the presence in the same agro-pedological context of olive trees (cultivars: Cellina di Nardò and Ogliarola) showing both tolerance (T) and non-tolerance (NT) to OQDS, and the soils beneath the tree canopy were collected. Different soil physical-chemical properties (texture, organic C, nutrients) were measured, and the microbial community was examined both regarding functions — through community-level physiological profiling (CLPP) using ECOPLATES — as well as taxonomy and community diversity, via targeted metagenomic sequencing of Bacteria (16S V3–V4) and Fungi (ITS2) using the MiSeq platform. Preliminary findings highlighted pedological variability between and within fields, paralleled by differences in microbial community diversity, particularly for fungi. The two cultivars showed distinct microbial assemblages, and differences also emerged between the communities associated with T and NT trees. These initial results suggest a possible association between soil microbiome and the tolerance mechanisms against *Xf*.

P28. The World Olive Germplasm Bank of cordoba: a source for research on *Xylella* control

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(See thematic session 1.6b)

P29. Metagenomic profiling of rhizosphere microbiota associated with *Xylella fastidiosa*-infected host plants

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The rhizosphere microbiome plays a significant role in plant adaptation, nutrient cycling, resistance to biotic and abiotic stresses, and symbiotic interactions. This study aimed to assess differences in the taxonomic and functional structure of the rhizosphere-associated microbiome between *Xylella fastidiosa*-infected and non-infected plants. Analyses were performed on potted plants of olive, grapevine and rosemary, artificially infected with strains of different subspecies, and on olive cultivars exhibiting varying responses to the bacterium (Ogliarola, Arbequina, Kolovi, Leccino). DNA from 53 soil samples of infected and control plants underwent shotgun Illumina sequencing. The resulting data were analysed using the CLC Genomics Workbench and SqueezeMeta pipelines for co-assembly, gene prediction, binning, taxonomic analysis, and comprehensive functional annotation. Over 90% of the microbiome consisted of bacteria, with

Proteobacteria, Actinobacteriota, Bacteroidota, Thermoproteota, and Halobacteriota as the most abundant phyla. Alpha diversity analysis showed a relatively stable microbial composition between infected and control plants in most cases, except for olive cv. Leccino and grapevine, where statistically significant differences were observed (p-value = 0.05 for both). Principal coordinate analysis based on Bray–Curtis dissimilarities demonstrated a convergence of microbial composition within each host type regardless of infection status, while distinct clustering was observed among different plant species. This suggests the microbial community is strongly affected by the recruiting host. Differential abundance analysis revealed a significant dominance of specific taxa associated with infection. Functional analysis indicated that the rhizosphere of infected plants exhibited enhanced pathways related to quorum sensing, stress tolerance, nitrogen fixation, and alternative carbon utilization. In contrast, non-infected plants were enriched in pathways associated with plant growth-promotion, antagonism, and secondary metabolite production. These findings provide insight into the role of the rhizosphere microbiome in promoting plant resilience under *Xylella* infection and highlight its potential application as a biomarker for plant health. Furthermore, the result suggests the possible use of functional microbes in the sustainable management of *Xylella*.

This work is part of the project REACH-XY (State Budget Law of Italy N. 234, 30/12/2021).

P31. Secondary metabolites from *Pseudomonas fluorescens* and *Bacillus velezensis* strains with antimicrobial activity against *Xylella fastidiosa* reducing its population in *Nicotiana benthamiana*

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The development of biopesticides based on microbial metabolites have received increasing attention to be included in the sustainable management of different plant diseases, including those caused by *Xylella fastidiosa*. Strains of *Bacillus* spp. and *Pseudomonas* spp. produce a wide diversity of antibacterial peptidic compounds that may represent alternatives to conventional pesticides, as some exhibit a broad spectrum of antimicrobial activity, low toxicity, and high biodegradability [1]. The present study was focused on the metabolites produced by *Bacillus velezensis* A17 and *Pseudomonas fluorescens* EPS894. The aims of this study were to evaluate (i) the in vitro antibacterial activity against *X. fastidiosa*, (ii) the efficacy in reducing *X. fastidiosa* population in inoculated Nicotiana benthamiana plants, (iii) and to identify the compounds responsible for this activity. Metabolites were isolated from the cell-free supernatants by a solid phase extraction procedure. The obtained fractions were evaluated for their antagonistic activity against *X. fastidiosa* subsp. *fastidiosa* (IVIA5387) and *X. fastidiosa* subsp. *multiplex* (M12) through growth inhibition in vitro assays using Bioscreen C (antibacterial activity) and contact tests combined with culturable plate counting (bactericidal activity). Then, the biocontrol efficacy of two active fractions was determined against three subspecies of *X. fastidiosa* (*fastidiosa*, IVIA 5387; *multiplex*, M12; and *pauca*, DeDonno) in inoculated N. benthamiana plants under greenhouse conditions. Both fractions reduced the population levels of IVIA 5387 and DeDonno in the xylem of plants in comparison with the non-treated control. However, none of the fractions reduced significantly the population levels of M12. In addition, the active fractions were characterized by Liquid Chromatography-Tandem Mass Spectrometry (UHPLC/QTOF-MS) to identify the compounds responsible for the activity. In summary, metabolites from B. velezensis A17 and P. fluorescens EPS894 showed antimicrobial activity against *X. fastidiosa* reducing its population in *N. benthamiana*.

[1] Montesinos, E. 2023. Functional peptides for plant disease control. Annu. Rev. Phytopathol. 61:301–24.

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P32. Antisense peptide nucleic acids (PNAs): a promising novel strategy to control *Xylella fastidiosa*

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Xylella fastidiosa (*Xf*) is a Gram-negative phytopathogenic bacterium responsible of several plant diseases in economically relevant crops such as almond, olive, grapevine and citrus trees. Currently, there is not any effective treatment to cure the infected plants from *Xf*. Therefore, it is necessary to develop new strategies aligned with the European environmental regulations to mitigate the impact of the diseases [1]. The Peptide Nucleic Acids (PNAs) are nucleic acid analogs where the sugar-phosphate backbone is replaced by 2-aminoethylglycine repeating units that incorporate the nucleobase through a methylene carbonyl linkage. Due to its capacity to hybridize specifically, strongly, and stably with nucleic acids through base pair, and for being resistant to nucleases and proteases, PNAs have been described as good candidates for antisense therapy [2]. Thus, the main objective of this work was to design and synthetize PNAs targeting the acpP essential gene in *Xf* to inhibit the bacterial growth through antisense strategy.

Firstly, the antisense sequence targeting the acpP gene in *Xf* was designed. Additionally, a cell-penetrating peptide (CPP) and a spacer were included in the design to enhance the cellular uptake and improve its solubility, respectively. Four different PNA-CPP conjugates were designed. Moreover, mismatch and scrambled PNA sequences were included to verify the specificity of the hybridization. The conjugates were designed and obtained by solid-phase synthesis using a Fmoc/tBu strategy, purified with reversed-phase column chromatography, analyzed by HPLC, and characterized through mass spectrometry (ESI-MS). Finally, the conjugates' growth inhibition activity against *Xf* was assessed in vitro.

[1] M. Mourou, G. Incampo, M. Carlucci, D. Salamone, S. Pollastro, F. Faretra and F. Nigro Front. Plant Sci. 2025, 16:1608687.

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P33. Optimizing in vitro regeneration and transformation in *Olea europaea L.* to enable efficient genome editing to combat *Xylella fastidiosa*

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Environmental constraints and the emergence of newly introduced pests and pathogens increasingly threaten crop productivity and food security. In olives (*Olea europaea L.*), the primary menace is the broad host-range pathogen *Xylella fastidiosa*. Given the species' long generation time, in vitro tissue culture, genomic assisted breeding, transformation and regeneration are crucial to use genome editing to introduce targeted mutations. This approach depends on robust protocols for regenerating improved plants from single cells or complex explants. In this research, we optimized tissue culture and transformation workflows to enable rapid improvement. An in vitro collection of olive cultivars was established following BOKU protocols (Joseph et al., 2023; Burillo Cartagena, 2024). Callus induction was evaluated across four media, and rooting responses were evaluated under varying auxin concentrations. To enhance transformation efficiency, we tested the effect of a one-week acclimation period prior to Agrobacterium-mediated co cultivation, with GUS assays used to confirm successful transformation events.

Three of the four media supported high callus induction rates (96–98%), although only one medium produced callus with morphogenetic potential. Rooting responses were strongly genotype dependent and require further refinement. A one-week acclimation period improved callus development under selection during Agrobacterium assays, and GUS staining verified transformation events. Ongoing work focuses on optimizing rooting, inducing somatic embryogenesis, and establishing protoplast regeneration, all critical steps toward enabling efficient genome editing pipelines in olive.

Keywords: *Olea europaea*, *Xylella fastidiosa*, tissue culture, callus induction, rooting, Agrobacterium-mediated transformation, somatic embryogenesis, protoplasts, climate constraints.

Burillo Cartagena A.P. 2024. In Vitro Culture Techniques for Genetic Transformation of Olive: A Step Towards Xylella fastidiosa Resistance Breeding. MSc thesis. Vienna: University of Natural Resources and Life Sciences.

Joseph, J. 2023. Biotechnological Approaches to Resistance Breeding in Olea europaea. MSc thesis. Vienna: University of Natural Resources and Life Sciences

P34. Top-grafting ancient olive trees as preventive measure against olive quick decline syndrome: preliminary field evidence

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(See thematic session 1.6a)

P35. Pruning history shapes *Xylella fastidiosa* subsp. *pauca* colonization and endophytic microbiome dynamics in susceptible and tolerant olive cultivars.

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One of the most critical challenges currently affecting agriculture is posed by *Xylella fastidiosa* subsp. *pauca* ST53 (*Xfp*), the vector-borne bacterial pathogen responsible for olive quick decline syndrome (OQDS). In areas where the bacterium is already established, the adoption of an integrated disease management strategy, combining the use of resistant or tolerant cultivars, vector control, and optimized agronomic practices, may help limit pathogen spread. However, the extent to which agronomic interventions such as pruning influence both bacterial colonization and the endophytic microbial communities of olive trees remains poorly understood. The aim of this study was to evaluate, in naturally infected olive trees belonging to susceptible and tolerant cultivars, the effectiveness of different pruning cycles in limiting bacterial colonization by monitoring *Xfp* population dynamics through real-time PCR. In addition, the study investigated differences in the endophytic microbiota of olive trees subjected

to different pruning regimes or left unpruned by means of metagenomic analysis. The cultivar Cellina di Nardò showed high susceptibility to *Xfp*, with greater symptom severity and higher bacterial loads even after pruning. Only slight differences in symptom severity were observed between pruned and unpruned plants, indicating that pruning had a limited effect on disease management in this susceptible cultivar. By contrast, the cultivar Leccino exhibited genetic tolerance, maintaining low symptom severity and very low bacterial levels at all assessment dates. These findings highlight the key role of host genetic resistance in limiting bacterial colonization and disease development, whereas pruning appears to represent only a secondary management tool in Leccino because of its inherent resilience. Overall, the data indicate that pruning history influences not only the occurrence and relative abundance of *Xylella fastidiosa*, but also the broader microbial context in which the pathogen is embedded. These results support the hypothesis that differences in disease severity among treatments are associated with management-driven microbiome trajectories, with recent pruning linked to unstable and heterogeneous microbial communities potentially more permissive to symptom expression, and older pruning associated with more stable microbial assemblages that may modulate host–pathogen interactions over time.

P36. Plant cell wall dynamics in olive cultivars with contrasting responses to *Xylella fastidiosa*

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(See thematic session 1.6d)

P37. Genomic Insights into *Lecanicillium aphanocladii*, a biological control agent of *Philaenus spumarius*, through Comparative Analysis of Entomopathogenic Fungi

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Entomopathogenic fungi represent an important resource for sustainable pest management and biological control strategies. A comparative genomics study was conducted to investigate the genomic diversity and functional potential of entomopathogenic fungal species. The study focused on the genome sequence of a strain *Lecanicillium aphanocladii* strain with activities against *Philaenus spumarius* and its comparison with newly assembled and annotated genomes from 27 entomopathogenic fungal strains obtained from public databases. The dataset included representatives from several genera with recognized entomopathogenic activity, including *Lecanicillium*, *Beauveria*, *Cordyceps*, *Akanthomyces*, *Gamszarea*, *Parengyodontium*, *Samsoniella*, and *Simplicillium*. The *L. aphanocladii* genome was assembled de novo and structurally annotated using standardized genome assembly and gene prediction pipelines. Assembly quality assessment revealed a genome size of 36.51 Mbp distributed across 250 contigs, with a GC content of 51.71%. The assembly showed high continuity (N50 = 232 kb; L50 = 47) and completeness, with a BUSCO score of 97.3%. A total of 11,108 genes were predicted, including 10,978 coding sequences and 130 tRNA genes. Functional annotation included KOG classification, KEGG pathway mapping, CAZyme profiling, secretome prediction, secondary metabolite biosynthetic gene clusters (antiSMASH), and known fungal virulence factors. Comparative analyses encompassed ortholog clustering, phylogenomic reconstruction, and functional profiling of gene families involved in pathogenicity, host interaction, and environmental adaptation. This integrative comparative framework revealed conserved and lineage-specific functional traits among entomopathogenic fungi, including genes likely involved in insect infection, host colonization, and environmental resilience. These findings provide comprehensive genomic resources for identifying candidate determinants relevant for biological control applications and advance the development of environmentally sustainable pest management strategies.

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P38. Diffusible signal factor produced by transgenic *Citrus sinensis* plants modulates the plant-associated microbiome

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The colonization of plant hosts by *Xylella fastidiosa* (*Xf*) is modulated by diffusible signaling factor (DSF), a molecule involved in quorum sensing (QS). In previous studies, our research group developed genetically modified Citrus sinensis plants overexpressing rpfF (GM), which encodes the RpfF enzyme responsible for DSF synthesis. DSF overexpression interfered with bacterial signaling and promoted a "pathogen confusion" effect, inducing premature biofilm formation, reducing bacterial motility within the xylem, and consequently decreasing disease symptom severity. Because DSF is a QS molecule in many bacterial species, we investigated whether DSF produced by GM plants could also influence the plant microbiome. Roots from GM and non-transgenic control (WT) plants were subjected to metataxonomic analysis to evaluate differences in microbial community structure and abundance between the two genotypes. Betadisper analyses showed no differences in community dispersion between WT and GM plants, while LEfSe identified genotype-associated taxa, suggesting that transgenesis may not restructure the microbial community globally but could influence the relative abundance of specific microbial lineages within the community. In total, 42 and 13 exclusive amplicon sequence variants (ASVs) were identified in GM and WT plants, respectively. GM plants exhibited a higher representation of bacteria mainly belonging to the classes Alphaproteobacteria and Gammaproteobacteria, particularly members of the order Hyphomicrobiales. ASVs related to genera such as Steroidobacter and Brevundimonas, as well as members of the family Nitrosomonadaceae, were also detected. In contrast, WT plants presented exclusive ASVs associated with the genera Pseudomonas, Sphingobium, Rhizobium, Ensifer, Variovorax, and Cupriavidus. The production of DSF in GM plants appears to favor the proliferation of some microorganisms while disadvantaging others. Since DSF molecules act as QS signals that modulate bacterial physiological processes such as biofilm formation, motility, and microbial interactions, DSF production may alter microbial colonization dynamics. These results suggest that DSF production modulates the bacterial community associated with plants, promoting "pathogen confusion" that shifts microbiome composition and potential functional profiles.

P39. *Bacillus velezensis* n3.2 as a promising biocontrol agent *against Xylella fastidiosa* subsp. *pauca*

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The lack of effective strategies to control *Xylella fastidiosa* subsp. *pauca* (*Xfp*), the causal agent of olive quick decline syndrome in the Mediterranean region, has prompted researchers to find solutions for its containment. This study aimed to evaluate the activity of the strain *Bacillus velezensis* N3.2, previously selected through an in vitro screening, for its antagonistic effects against the *Xfp* ST53 strain. Its antagonistic activity against *Xfp* was demonstrated in co-culture assays, along with the antimicrobial effects of its cell-free supernatants. To obtain insights on the genetic mechanisms associated with potential biocontrol activity of the strain, whole genome analysis was carried out. Genome analysis highlighted high similarity to known gene clusters associated with antimicrobial activity, induction of plant immunity, resistance to pathogens, plant growth promotion, and biofilm formation. Comparative genomics with other *B. velezensis* strains, known for antagonistic activities against several plant pathogens, showed that a set of biocontrol related genes are conserved in *B. velezensis* species, while others appear to be strain specific. Notably, N3.2 strain genome analysis revealed the potential capability of producing several secondary metabolites with specific antimicrobial properties. To validate these predictions, the exogenous metabolome of strain N3.2 was characterized using high-resolution mass spectrometry (HRMS) coupled with liquid chromatography (LC). Preliminary results confirm the production of broad range of secondary metabolites including those yet reported for their antimicrobial activity. Ongoing greenhouse experiments, under controlled environmental conditions, are elucidating the impact of strain N3.2 on olive plant growth and its capability to colonize host tissues. The results demonstrate the endophytic behavior of the strain N3.2 and its stable colonization of olive plants. Overall, our findings indicate that *B. velezensis* N3.2 is a promising candidate for the biocontrol of *Xfp*.

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P40. Biofilm biomineralization induced by *Xylella fastidiosa*

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Xylella fastidiosa (*Xf*) is a pathogenic bacterium that affects agricultural commodities worldwide under diverse environmental conditions, causing impacts ranging from mild to severe. *Xf* biofilms block xylem vessels, disrupting water and nutrient transport in affected plants. While xylem vessel occlusion is typically viewed as pathogen-controlled and host-dependent, our current work aims to re-examine this paradigm. We show that the physicochemical conditions around *Xf* cells influence cell aggregation and biofilm development, thereby identifying environmental pressure as a key factor for disease progression. We have specifically experimented with the xylem sap calcium-to-phosphate ion (Ca/P) ratio, glutathione as a redox modulator, and pH. Separately or together, these factors affected *Xf* cell aggregation and biofilm formation. We also determined that cell-surface properties are highly dependent on chemistry rather than relying on biological specificity. A xylem-mimicking medium enriched with CaCl2, phosphates, and reduced glutathione (GSH) not only stimulated a robust *Xf* biofilm growth, but also led to the formation of carbonates and hydroxyapatite, a calcium phosphate complex molecule, as the medium pH was increased from 6 to 7 and 8. Altogether, this indicates that calcification is the final step in biofilm maturation. Our findings suggest that *Xf* pathogenicity may result from the plant's inability to effectively respond to multiple abiotic stresses associated with the infection process. Plants that are naturally stress-resilient or genetically engineered to achieve such resilience are more likely to prevent *Xf* diseases. Overall, our data suggest that disease progression may be reversible under appropriate chemical and pH conditions.

P41. Integrated metagenomics, metabarcoding and culturomics reveal beneficial bacteria in the xylem microbiome of the resistant olive cultivar Leccino

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(See thematic session 1.6e)

P42. Effects of diffusible signaling factor overproduction in *Citrus sinensis* on vector behavior and acquisition of *Xylella fastidiosa*

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The transmission *of Xylella fastidiosa* relies on the successful colonization of host plants and acquisition by xylem-sap-feeding insect vectors, processes regulated by quorum sensing mediated by diffusible signal factors (DSFs) produced by the RpfF enzyme. In this study, we investigated whether DSF overproduction in transgenic sweet orange (Citrus sinensis cv. ‘Hamlin’) could influence interactions between the pathogen and its insect vector. Transgenic plants overexpressing the rpfF gene, grafted onto ‘Rangpur lime’ rootstocks, were evaluated through settling preference assays, electrical penetration graph (EPG) recordings, and bacterial acquisition experiments using the sharpshooter *Macugonalia leucomelas*, an important vector of *X. fastidiosa*, the causal agent of Citrus Variegated Chlorosis (CVC). Both DSF-producing plants and bacterial infection reduced insect landing preference. Moreover, insects feeding on transgenic plants exhibited shorter periods of xylem sap ingestion compared with those feeding

on non-transgenic control plants. These behavioral changes were associated with a 15-percentage-point reduction in bacterial acquisition by insects feeding on infected transgenic plants. Overall, our findings demonstrate that DSF overproduction in citrus can modify vector landing and feeding behavior at stages critical for pathogen acquisition, highlighting vector responses as an important component of the interaction between DSF-producing citrus plants and *X. fastidiosa* within the CVC pathosystem.

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P43. Characterization and monitoring of metabolic profiles of resilient olive trees infected by *Xylella fastidiosa* subsp. *pauca*

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Xylella fastidiosa subsp. *pauca* is responsible for the “olive quick decline syndrome” (OQDS) on the Salento and several other areas of the Apulia region (Italy), causing severe economic losses to olive groves [1]. In this study, a non-target 1H Nuclear Magnetic Resonance (NMR) Spectroscopy fingerprint approach, followed by Multivariate Statistical Analysis (MVA), was applied to observe the possible changes of metabolic profiles in leaf extract samples (as proxy for xylem) of resilient trees in different areas of Salento. Samplings were carried out in Brindisi, Gallipoli, Otranto and S.M. di Leuca areas. 1H NMR spectra revealed a complex pattern of signals due to sugars, phenolic compounds and aldehydic secoiridoids. MVA showed differences in metabolic profile of xylem extracts according to sampling area. Mannitol, oleuropein and its aldheydic form were the main metabolites responsible for the observed differences. Moreover, in this study, NMR combined with MVA was also applied to investigate the metabolic pattern of olive oils obtained from resilient olive trees of Brindisi and Gallipoli area. The results showed as the geographical origin mainly influences the olive oil minor components (polyphenols). In particular, the olive oil samples from Brindisi were characterized by higher relative content of polyphenols with respect to Gallipoli. In conclusion, both, leaf extract and olive oil samples of investigated resilient trees show differences in their metabolic profiles, which appear geographically correlated and could be discussed in term of plant response according to space and time progression of the disease.

[1] Serio, F., Imbriani, G., Girelli, C. R., Miglietta, P. P., Scortichini, M., & Fanizzi, F. P. (2024). A decade after the outbreak of Xylella fastidiosa subsp. pauca in Apulia (Southern Italy): Methodical literature analysis of research strategies. Plants, 13(11), 1433.

P44. Phylogenomic Placement and Subspecies Classification of a *Xylella fastidiosa* Isolate from the Amazon, Brazil

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Subspecies-level classification within *Xylella fastidiosa* is critical for epidemiological surveillance and phytosanitary risk assessment. We performed genomic characterization of the first sequenced isolate from the Brazilian Amazon using Average Nucleotide Identity (ANI), multilocus sequence typing (MLST), and core genome phylogeny. ANI was calculated using FastANI (v1.34) against representative complete genomes of subspecies *pauca* (9a5c), *fastidiosa* (Temecula1), *multiplex* (M12), and *sandyi* (Ann-1). Phylogenomic analysis included 41 representative *Xylella* genomes encompassing the diversity of the genus and its major subspecies, with *Xanthomonas* species used as outgroups. Genomes were reannotated with Prokka (v1.15.6) and the pangenome reconstructed using Panaroo (v1.6.0) under strict parameters. The core genome (≥95% presence) comprised 272 genes (291,339 nucleotide sites). Maximum likelihood inference was performed with IQ-TREE (v3.0.1) (GTR+F+I+R4 model), with branch support from 1,000 ultrafast bootstrap replicates. The isolate showed 99.39% ANI with reference strain 9a5c and approximately 96% identity with representatives of other subspecies, confirming assignment to *X. fastidiosa* subsp. *pauca*. In silico MLST classified the isolate as sequence type ST11, a lineage associated with Brazilian citrus strains. Phylogenomic analysis positioned the Amazonian isolate within the Brazilian *pauca* clade, clustering with ST11 citrus strains with high bootstrap support. No Amazon-specific lineage was detected. These findings confirm the subspecies and sequence type assignments and improve our understanding of the geographic distribution of *Xylella fastidiosa* subsp. *pauca* in Brazil.

P45. Relevance of genomic data to infer the evolutionary history of *Xylella fastidiosa* in Europe

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Advancements in the accessibility and cost-effectiveness of genomic technologies have significantly expanded the availability of whole-genome sequences, empowering high-resolution phylogenomic approaches to gather insights into pathogen dispersal, introduction events, and transmission dynamics. For instance, the number of sequenced isolates of *Xylella fastidiosa* (*Xf*) has grown considerably following its recent expansion into Europe, driven by the need to elucidate introduction pathways and dispersal patterns. However, the robustness of these computational reconstructions critically depends on the availability of comprehensive and geographically representative sequenced isolates. In this work, we focused on two of the most recent *Xf* records in Europe, the Apulian outbreaks, associated with the occurrence of isolates belonging to the subsp. *multiplex* harboring the sequence type ST26, and with isolates of the subsp. *fastidiosa*, ST1. Due to the very limited genomic resources available for the ST26-isolates, the dataset was further implemented by sequencing isolates from infected plums in Brazil. The analyses, while confirmed the close genetic relatedness of the Apulian and Brazilian isolates, allowed to extend the European sequenced isolates of the subsp. *multiplex*, which although displays the widest genetic diversity and distribution in Europe, it is still underrepresented in terms of available genomic sequences. Conversely, for the subsp. *fastidiosa* the availability of a worldwide large dataset of ST1 and ST2 genomes, allowed us to perform molecular clock analysis to infer, using a Bayesian approach, their origin and time of introduction. Although the statistical confidence of the estimation rates remains constrained by limited sampling and insufficient temporal signal, preliminary results would suggest a relatively recent introduction of this subspecies in the region. Overall, expanding the genomic landscape of *Xf* through the availability of high-quality genomes, particularly from newly detected outbreaks, represents a crucial step toward improving phylogeographic resolution, and to help pinpoint the timing and origin of independent introductions into Europe, thereby providing critical evidence for phytosanitary surveillance and risk assessment strategies.

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P46. Influence of endophytic colonization by *Metarhizium brunneum* on host-plant preference of spittlebugs (*Neophilaenus campestris* and *Philaenus spumarius*)

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The first system included Sonchus oleraceus L., the fungal strain EAMa 01/58-Su, and the spittlebug *Philaenus spumarius* L. The second system included *Lolium rigidum Gaud*., the fungal strain EAMa 10/01-Fil, and *Neophilaenus campestris* (Fallen). Choice experiments were conducted under semi-controlled conditions to assess the response of adult spittlebugs to *M. brunneum* colonized plants. Plants were sprayed with a conidial suspension of *M. brunneum* (1×10^{8} conidia mL^{-1}), while control plants were treated with sterile water containing 0.01% Tween 80. After 48 h incubation, eight plants (four treated and four control) were placed in mesh cages in an alternating circular arrangement. Twenty-four adult insects were released in the center of each cage and their position on plants was recorded after 1, 2, 3, 4, 24 and 48 h. At the end of the experiments, plants were analyzed for endophytic colonization, obtaining 60.0 ± 4.1% in *M. brunneum* treated plants. In the S. oleraceus–EAMa 01/58-Su–*P*. spumarius system, no significant differences in insect preference were detected. Conversely, in the *L. rigidum*–EAMa 10/01-Fil–*N. campestris* system, a significant initial preference for EEF plants was observed at 1 and 2 h after release ($\chi^2_1 = 10.97$, $p = 0.00$; $\chi^2_1 = 5.78$, $p = 0.01$). However, as exposure time increased, insects actively explored the experimental arena and eventually settled and fed indiscriminately on both treated and control plants, with no significant differences detected from 3 to 48 h. These findings could be considered in the development of push–pull or attract-and-infect strategies using *M. brunneum* colonized cover plants in olive agroecosystems.

P47. Elucidating Overlooked Aspects of the Tripartite Interaction *Philaenus spumarius-Xylella fastidiosa*-Host Plant for a Sustainable Bacterium Control

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Xylella fastidiosa subsp. *pauca* (*Xfp*) spread in European olive orchards is primarily driven by vector activity, i.e., the abundance of insect vectors and the time they spend on olive plants. Theoretically, increased plant suitability leads to longer vector permanence and, consequently, a higher likelihood of pathogen transmission. Currently, there is a knowledge gap regarding the plant traits mediating host plant location and acceptance by the meadow spittlebug *Philaenus spumarius* (*Ps*), the main European vector of *Xfp*. Therefore, this work aimed to investigate the drivers of host plant acceptance by *Ps*., a pivotal step in *Xfp* transmission. First, host preference was assessed among olive varieties differing in susceptibility to *Xfp*. Using choice tests, survival analysis, Electropenetrography (EPG), microscopy, and xylem primary metabolite profiling, we compared the susceptible variety Ogliarola Salentina with the tolerant genotypes Leccino and FS-17. Spittlebugs showed a significant preference for Ogliarola Salentina. These behavioral differences were primarily explained by variation in xylem sap chemistry rather than vascular anatomy, indicating that xylem chemistry plays a dominant role in spittlebug host acceptance. Subsequently, the chemical determinants of host acceptability were investigated by linking spittlebug probing behavior to the xylem sap metabolite composition of different olive varieties. The analysis revealed that host acceptance is not driven by single compounds but by the overall metabolic profile of the xylem sap. In particular, varieties characterized by higher concentrations of organic acids were less suitable for sustained feeding, suggesting a collective deterrent effect. Finally, given that resistance traits to spittlebugs may exist within olive germplasm, host plant suitability was evaluated across 14 olive varieties widespread in Apulia, Southern Italy. Some genotypes, including Leccino, Pendolino, and Arbequina, exhibited traits that reduced vector acceptance and permanence, largely associated with unfavorable xylem chemical profiles. Overall, this work demonstrates that xylem sap chemistry is a pivotal factor underlying host plant acceptance by *Ps*. These findings open new perspectives for *Xfp* management through the selection of germplasm less suitable to the vector or via agronomic practices aimed at manipulating xylem chemistry to reduce plant suitability and vector activity.

P48. Fungal culturome of olive trees infected with *Xylella fastidiosa* subsp. *pauca* reveals potential antimicrobial activity

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The spread of *Xylella fastidiosa* subsp. *pauca* (*Xfp*) represents one of the most significant threats to olive (*Olea europaea L.*) cultivation in Apulia. Since 2014, in the absence of effective control measures, a targeted olive tree eradication programme has been implemented to mitigate the epidemic's progression. In recent years, management efforts against *Xfp* have increasingly focused on improving knowledge of the xylem environment and its associated microbiome to identify microbial antagonists capable of occupying the ecological niche of *Xfp*. The present study aimed to isolate endophytic fungi from xylem sap and evaluate their potential antagonistic activity against *Xfp*. More than 100 *Xfp*-infected olive tree samples were collected from different areas of Apulia. To selectively isolate microorganisms inhabiting the xylem, twigs were surface-sterilized and then divided into two portions: one end was crushed with sterile forceps, whereas the other was gently pressed onto buffered charcoal yeast extract (BCYE) agar. After 25 days of incubation, fungal colonies were recovered and identified by molecular analysis. The isolates were subsequently cultured in Czapek broth for 60 days. The resulting culture filtrates were passed through a 0.22 µm membrane filter and tested for in vitro activity against *Xfp* using a well diffusion assay. Notable inhibitory activity was detected in 4 of the 20 isolates, belonging to the genera Aspergillus and Libertasomyces, which produced inhibition zones ranging from 30 to 40 mm. Further studies are in progress to identify the active compounds present in these culture filtrates and to clarify the ecological role of these endophytic fungi within the xylem niche.

P49. A sustainable approach against *Xylella fastidiosa*: perceptions, knowledge, and obstacles to the use of thermal treatments in European nurseries

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Within the European research project BeXyl (WP 3), sustainable solutions are being explored to guarantee healthy propagation material. A Hot Water Treatment is already authorized by EU Regulation 2020/1201 for *Vitis* species to ensure plants are free from *Xylella fastidiosa*. This sustainable option could potentially be expanded to a broader range of plant species. With this objective, a structured, voluntary, and anonymous questionnaire was developed. The survey aims to assess the nursery sector's level of knowledge on *Xylella*, analyze the interest in adopting thermal treatments for plant health, identify the main technical and economic barriers to their implementation, determine support needs to guide research efforts and future regulatory developments. The questionnaire was available in several languages and distributed across European countries, focusing on those affected by *Xf*, like France, Italy, Portugal and Spain, assessing nursery characteristics, experience with *Xylella* damage, awareness of thermal treatments, and future adoption potential. The earliest results show that awareness of the pathogen is uneven across the sector. Specific familiarity with thermal methods (e.g. hot water treatment) for plant health control is generally medium-low across the European area, with most respondents stating they are "not familiar at all" or only "somewhat familiar". Although many respondents recognize the value of the method as environmentally friendly and useful for reducing chemical use, practical adoption in the nurseries remains hindered by significant barriers, such as the high initial investment in equipment, a lack of specific technical knowledge, uncertainty about actual effectiveness, and the fear of causing potential damage to the plants. To successfully implement these treatments, operators consider financial incentives (e.g., subsidies and grants) essential, accompanied by technical training, workshops, and more precise information on the technology. Finally, there is a broad and clear consensus in favor of promoting certified "*Xylella*-free" plants within the sector. Thermal treatments are perceived as a valid, eco-sustainable prospect, but the transition is currently blocked by economic and knowledge obstacles. The introduction of targeted financial incentives and clear educational initiatives will be crucial to reassure producers about the efficacy and safety of the treatments, thus favoring the spread of protected and resilient nursery supply chain.

P50. Diverse and abundant *Xylella fastidiosa* genotypes associated with olive and other tree hosts in Southern California

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Bacterial leaf scorch is commonly observed in Southern California urban forests, but existing knowledge on the *Xylella fastidiosa* (*Xf*) genotypes circulating in this region is scarce. Although initial reports document a wide range of symptomatic host plant taxa, there is limited insight into the diversity, host range, and distribution of *Xf* strains in Southern California urban forests despite contemporary reports of severe disease. A systematic field survey was conducted in San Diego, Los Angeles, and Orange counties to characterize *Xf* diversity and spatial distribution in urban trees. Six hundred sixty-three symptomatic trees were sampled in the summer months of 2023-2025. Sweetgum (*Liquidambar styraciflua*), purple-leaf plum (*Prunus cerasifera*), Pohutukawa (*Metrosideros excelsa*), and olive (*Olea europaea*) were the focal hosts of this study due to frequent observations of putative disease symptoms and their general prevalence in urban forests. MLST was implemented for samples that tested positive for *Xf* (sequencing from plant DNA extractions). A cross-inoculation trial began in the spring in 2025 where all four focal host species were infected with *Xf* strains from their respective hosts and from the additional host species. Approximately 80% of the symptomatic trees tested positive for *Xf*, including 86% of 263 olives. Two subspecies of *Xf* were observed (*Xf* subsp. *multiplex* and *Xf* subsp. *sandyi*), and at least 9 discrete sequence types (STs) were detected among the four focal host species, including 7 novel ST. A high degree of host overlap was observed within STs. Only two *Xf* isolation attempts from Pohutukawa and purple-leaf plum were successful. No symptoms were documented in the first year of greenhouse inoculations with one exception: a field isolated subspecies multiplex strain from Pohutukawa (MEEX_33, ST6) caused severe leaf scorch symptoms in the grapevine control. These findings suggest *Xf* is far more abundant and diverse in Southern California urban forests than previously reported.

P51. Global analysis of the genetic diversity of *Xylella fastidiosa* and its relationship with host range and geographic distribution

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The large genetic and biological diversity of *Xylella fastidiosa* (*Xf*) is extensively documented, leading to a classification into several subspecies and 90 different sequence types (STs). An extensive review of evidence from scientific literature and databases was carried out to build a dataset designed to identify correlations between different subspecies/STs and their geographic distribution and host range. Analysis of the geographic distribution aimed to assess the extent of genetic diversity currently detected in EU compared to non-EU regions. Phylogenetic reconstruction based on MLST and whole-genome analyses (249 complete genomes) consistently resolved *Xf* into three main clades corresponding to subsp. *fastidiosa*, *multiplex* and *pauca*, while *sandyi* and *morus* fell within the broader fastidiosa lineage, supporting the proposed taxonomic revisions. The Americas emerged as the primary center of global diversity, whereas EU outbreaks represent a markedly narrower subset of this variability. Amongst the 90 STs so far reported, four have been recorded exclusively in the EU, while 77 STs occur solely outside the EU, indicating a large external reservoir of diversity. Even so, all subspecies have been reported in EU represented by isolates characterized either in outbreaks or intercepted consignments. Infections in EU are dominated by few ST–subspecies combinations: ST53 -*pauca*, infecting olives and causing the severe decline syndrome; ST6/ST7-*multiplex*, infecting *Prunus* spp. and several ornamentals; and ST1-*fastidiosa*, detected mainly in grapevine and *Prunus* spp. Perennial species account for the majority of the natural infections recorded worldwide: olives and *Prunus* spp. dominate EU records, whereas grapevine, *Prunus* spp., citrus, and coffee prevail in the American continent. Several plant species can host multiple subspecies, potentially serving as epidemiological bridges and acting as hotspots for inter-subspecific recombination. Disease associated with the EU records refer mainly to the olive decline syndrome caused by ST53-isolates, leaf scorch in *Prunus* spp. and Mediterranean shrubs caused by ST6- and ST7-isolates, and Pierce's disease in grapevine caused by ST1-isolates. The implemented dataset provides an updated and detailed overview of the global and EU distribution and host association of *Xf* subspecies and STs. This work was carried out in the framework of the SPECIFIC AGREEMENT No 07-2025 - PARTNERSHIP AGREEMENT No GP/EFSA/PLANTS/2022/02-02. Amongst the 90 STs so far reported, four have been recorded exclusively in the EU, while 77 STs occur solely outside the EU, indicating a large external reservoir of diversity. Even so, all subspecies have been reported in EU represented by isolates characterized either in outbreaks or intercepted consignments. Infections in EU are dominated by few ST–subspecies combinations: ST53 - *pauca*, infecting olives and causing the severe decline syndrome; ST6/ST7-*multiplex*, infecting *Prunus* spp. and several ornamentals; and ST1-*fastidiosa*, detected mainly in grapevine and *Prunus* spp.

P52. Histological analysis of *Xylella fastidiosa* infection in Quercus pyrenaica in Northern Portugal

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Quercus pyrenaica Willd thrives in the intermediate zone between the Mediterranean sclerophyllous and the temperate deciduous forest. In December 2022, the presence of the bacteria *Xylella fastidiosa* (*Xf*) was confirmed in samples collected from a *Quercus pyrenaica* located in Sabrosa, Vila Real, Portugal. Following *Xf* infection, the transport of water and nutrients is hindered due to the occlusion of xylem vessels. This loss of hydraulic conductivity may lead to vessel blockage and subsequent embolism formation. The objective of this study was to investigate the interaction between *Xf* and Quercus pyrenaica tissues, as well as the mechanism by which the bacteria can spread through the plant's xylem vessels, ultimately resulting in the formation of vascular plugs. At the time of the sample collection (10 months post-detection), symptoms of Bacterial Leaf Scorch (BLS) began to appear. Examination of xylem vessels using both light and scanning electron microscopy (SEM) revealed the presence of various types of occlusions, predominantly tyloses. Additionally, fibrillar networks, gums, starch grains, and crystals were observed. The stem vessels exhibited significantly more occlusions compared to the leaves. Furthermore, individual bacterial cells were observed to be attached to the vessel wall. This implies that occlusions were primarily induced by tyloses and gums as a defensive response to the invasion of vascular pathogens, in addition to the pathogen itself. This study highlights the presence of starch grains in stems, which may function as a refilling mechanism, thereby preventing the loss of hydraulic conductivity in plants and potentially acting as a means to entrap the bacteria. These mechanisms exemplify the constitutive defense systems of the plant against *Xf*. Understanding the interaction between *Xylella fastidiosa* and *Quercus pyrenaica* is crucial, given that the latter species occupies nearly 95% of the natural distribution area of Portugal.

P53. Exploring the potential of Rep-WH1 prion-like proteins in the control of the phytopathogen Xylella fastidiosa

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(See thematic session 3.7C)

P54. Host genetic diversity does not prevent severe epidemics of generalist pathogens

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Host genetic diversity is widely assumed to buffer plant populations against emerging epidemics. While this prediction is supported in annual crop mixtures, it remains largely untested in perennial systems. We examined this question using the invasion of *Xylella fastidiosa* (*Xf*), a vector-borne xylem-limited bacterium causing almond leaf scorch disease (ALSD), introduced into Mallorca around 1993. Despite high intraspecific diversity among almond varieties, a severe epidemic developed across the island. Between 2017 and 2024, ALSD incidence and severity were monitored in three germplasm banks and three commercial orchards. A total of 10,370 samples from 110 varieties were analysed by qPCR and symptom scoring; 44% tested positive. Traditional local varieties (n = 88) were infected significantly more frequently than modern commercial cultivars (n = 22) ($\chi^2 = 511.28$, $p < 2.2 \times 10^{-16}$). This effect persisted after accounting for spatial heterogeneity: mixed-effects logistic models including field as a random factor confirmed variety origin as a strong predictor of infection probability ($p < 0.001$). Symptom severity was strongly negatively correlated with Ct values ($R^2 = 0.953$), indicating higher bacterial loads in more affected trees. Temporal infection dynamics differed markedly among varieties. A binomial mixed-effects model detected a significant interaction between variety and time ($\chi^2 = 34.61$, $p = 0.00029$), revealing strong genetic heterogeneity in susceptibility. Susceptible landraces showed steep increases in infection probability, whereas tolerant varieties displayed flatter trajectories and often remained uninfected after prolonged

exposure. Using a validated SEIR model calibrated with empirical data, we reconstructed epidemic trajectories from 1993 to 2024. Infection followed a sigmoidal growth curve characteristic of *Xf* outbreaks. Simulations assuming homogeneous planting of traditional varieties predicted 91% prevalence after 30 years, closely matching the current empirical estimate (~85%). In contrast, simulations assuming exclusive planting of 22 modern cultivars projected a maximum prevalence of 28%, and selecting 12 highly tolerant varieties would maintain prevalence below 5%. These results demonstrate that high host genetic diversity alone does not prevent severe epidemics when susceptible genotypes dominate. However, strategic deployment of tolerant varieties could substantially reduce long-term disease impact.

P55. Aggressiveness of subspecies and STs of *Xylella fastidiosa* in the surrogated host *Nicotiana benthamiana*

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The aggressiveness of seventeen *X. fastidiosa* strains isolated from diverse outbreaks, representing different subspecies and sequence types (STs), was evaluated in the surrogate host *N. benthamiana* by means of the analysis of the population levels, symptoms dynamics, dose-effect relationships and the transcriptomic response of the plant. All strains successfully colonized plants after micro-injection with a calibrated suspension, within 7 days post inoculation (dpi), with initial symptoms appearing after 14 dpi. Differences in patterns of population dynamics and disease progression were observed between strains. However, no direct relationship was found between bacterial population levels and symptoms severity, nor between strains belonging to the same subspecies and STs. Dose-effect relationships of the strains IVIA 5387, DeDonno, CN28 and GP18 showed a typical S-shaped dose-effect curve, establishing a minimum infective dose (MID) of less than 300 CFU per plant. The plant response was further studied for the subsp. *pauca* ST53 strains (DeDonno, CN28, and GP18) through the expression of 19 defense-related genes over a period of 30 days. Gene modulation was both strain and time-dependent. CN28 triggered the strongest gene overexpression (12 out of 19 genes), whereas DeDonno induced the lowest response (8 of 19 genes). At 4 dpi, all strains upregulated in *N. benthamiana* the genes PR1, PR1a and ERF1 and downregulated the PDF1.2 gene, correlated with an initial recognition of the pathogen and the activation of the salicylic acid (SA) defense pathway. At 30 dpi most of the genes were downregulated, particularly pathogenesis related (PR) genes, suggesting immune evasion by the pathogen. Our findings provide valuable insights into the interaction between *X. fastidiosa* and its hosts, highlighting the need to consider strain-specific behavior when designing disease management strategies.

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P56. Nanotrapping *Xylella fastidiosa*: Morphophysiological, Molecular, and Hyperspectral Evaluations to Uncover Mechanistic Efficacy and Disease Reduction Potential of Cellulose Nanocrystal Endotherapy

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(See thematic session 2.7d)

P57. Genetic diversity of *Xylella fastidiosa* in the French region Occitania

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(See thematic session 3.7d)

P58. Preliminary evaluation of the antimicrobial activity of olive oil industry by-products on *Xylella fastidiosa* subsp. *pauca*

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The olive oil industry generates several by-products that represent versatile and biologically valuable matrices, among which olive mill wastewater (OMWW) is particularly significant. OMWW is rich in bioactive molecules that, if properly recovered and stabilized, may find valuable applications in multiple sectors, including plant protection and sustainable crop management. In this study, three OMWW-derived powders were produced by spray drying. Two formulations were further enriched with blood orange (OMWW-A) and bergamot juices (OMWW-B) to explore potential synergistic interactions between olive- and citrus-derived phenolic compounds. The resulting powders were then tested in vitro against *Xylella fastidiosa* subsp. *pauca* (*Xfp*) ST53 to assess their antibacterial activity. Chemical characterization was performed by LC–MS/MS, providing qualitative and quantitative profiles of the phenolic composition. Among the tested formulations, OMWW contained the highest concentrations of olive-derived phenolics. Notably, lipophenols were also detected in this matrix. These compounds represent a relatively recently investigated class of amphiphilic phenolics that has attracted increasing attention because their structure may enhance bioavailability compared with more polar phenolic molecules. In contrast, OMWW-A and OMWW-B resulted enriched in citrus-derived flavonoids with OMWW-B exhibiting a more hybrid profile. Preliminary antibacterial activity against *Xfp* ST53 was evaluated using agar diffusion assays (disc and well diffusion). OMWW showed the most pronounced inhibitory effect in both methods. At a 1:2 dilution, a clear inhibition zone was detectable after 6 days, with mean diameters ranging from 2.5 to 2.75 mm. Inhibition increased over time, reaching 6.0–7.5 mm at 28 days. However, by day 35 the inhibition zones were no longer detectable, indicating loss of activity. At the lower concentration (1:10), inhibition zones were substantially smaller (approximately 2 mm at 6 and 28 days) and were absent by day 35. Control treatments consistently showed no antibacterial effect. Overall, these preliminary findings suggest that OMWW-derived phenolic concentrates can exert a moderate inhibitory effect against *Xfp*, particularly at higher concentrations, although the activity appears to diminish over time under the conditions tested. The research activities were performed under the Project “AGRITECH” (Spoke 2) (DD MUR n. 1032, 17/06/2022).

P59. Detection of *Xylella fastidiosa* in olive trees from Aiuruoca, Minas Gerais (Brazil), under N-acetylcysteine (NAC) treatments.

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The infection caused by *Xylella fastidiosa* in olive trees is an increasing challenge that requires management options to minimize the damage from the disease. This study aimed to assess the effect of N-acetylcysteine (NAC) on diseased olive plants in Aiuruoca, Minas Gerais, Brazil. First, plants showing symptoms were identified in the field and tested by qPCR, which confirmed the presence of *X. fastidiosa*. In May 2025, the plants were pruned and began treatment with NAC through biweekly applications. Disease severity was monitored every two months, tracking the development and reappearance of symptoms. Plants treated with NAC exhibited less symptom re-emergence compared to before treatment, indicating a potential benefit of NAC in reducing symptoms in infected plants. These findings suggest that NAC could serve as a useful additional tool for managing olives affected by *X. fastidiosa*, but further research with more plants, varied doses, different evaluation periods, and combined management strategies is needed.

P60. Host plant use by spittlebug nymphs in agroecosystems: a field survey supporting targeted vegetation management

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Current strategies aimed at containing the spread of the vector-borne bacterium *Xylella fastidiosa* include ground cover removal and tillage in order to curb the population the juveniles of spittlebug vectors as *Philaenus spumarius* and *Neophilaenus campestris*. However, continuous tillage might harm soil, biodiversity, and ecosystem services. Here we present data on host plant use by juveniles of the two spittlebug species obtained through a survey carried out across 105 olive orchard plots in Apulia, South-East Italy. *Philaenus spumarius* juveniles were absent from species as Oxalis pes-caprae, *Astragalus hamosus*, *Mercurialis annua*, *Fumaria parviflora*, and *Muscari* spp., while *N. campestris* almost exclusively developed on Poaceae, avoiding most dicots. Host selection was species-specific rather than family-dependent, possibly reflecting differences in xylem chemistry and plant architecture. These findings provide empirical support for selective vegetation management: cultivating plant species unsuitable for spittlebug nymphs could reduce vector populations while preserving ground cover benefits, avoiding the ecological shortcomings of indiscriminate removal. However, causal mechanisms remain untested, and the efficacy of this approach in reducing pathogen spread needs experimental validation. Moreover, potential side effects on non-target organisms and ecosystem functions require careful ecological assessment before large-scale implementation.

P61. Rational discovery of an FtsZ-targeting compound inhibiting in vitro growth across *Xylella fastidiosa* subspecies

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(See thematic session 2.7b)

P62. Repeated subculturing induces cellular alterations and increased biofilm formation in *Xylella fastidiosa* strain ST53 isolated from olive

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Isolates of *Xylella fastidiosa* subsp. *pauca* genotype ST53, the causal agents of the olive quick decline syndrome, have been extensively investigated in Europe to disclose biological and genetic information modulating their aggressiveness and pathogenicity in olives. In vitro studies have clearly unravelled that the reference ST53-strain De Donno has distinctive features compared to the reference Temecula1, i.e. it produced significantly more biofilm, showed significantly higher settling rate, showed a more aggregative phenotype (hyper attachment phenotype), a significant reduced fraction of planktonic cells and it appeared to be not prone to natural transformation. The aim of this study was to evaluate phenotypic changes and potential alterations in virulence-related traits in an olive-infecting ST53-strain (Cist-18) after successive in vitro subcultures, as previously showed for *X. fastidiosa* strains isolated from sweet orange. The strain Cist-18 was subcultured twenty (S20) and fifty (S50) times at regular intervals on PD3 agar medium, and the recovered colonies used for different assessments. Colony morphology was found to differ from the wild type, particularly in S50, which produced opaque colonies, strongly attached to the medium. A live/dead assay revealed no significant differences in cell viability. However, microscopy observations suggested a reduction in cell size in S50 cells. Transmission electron microscopy unveiled structural alterations in both S20 and S50, including damages to the cell wall. In addition, S50 exhibited pronounced phenotypic changes such as irregular and smaller cell shapes, increased production of exopolysaccharides, and a potential increase in vesicle formation. Planktonic growth was similar in the three different media, with the highest growth observed in PD3 medium. However, the S50 strain exhibited increased biofilm formation in all tested media. To assess the biological relevance of these changes, the strains were inoculated into a susceptible olive cultivar Ogliarola salentina and will be evaluated for their ability to colonize the plant vessels and induce symptoms. Future work will include whole-genome sequencing of the S50 strain to identify potential genomic alterations compared to the wild type, as well as gene expression analyses targeting genes associated with biofilm formation, quorum sensing, exopolysaccharide production, and virulence.

P63. Effects of extreme temperatures on growth, survival and recovery of *Xylella fastidiosa*

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Temperature plays a key role in the biology, ecology and epidemiology of *Xylella fastidiosa* (*Xf*). Although *Xf* has demonstrated a broad optimal temperature range and adaptability to diverse environmental conditions, the effect of extreme thermal events on survival, persistence, and the epidemiological implications remain poorly studied. This work evaluated whether different temperature treatments (TTs), applied at varying exposure times, exert lethal or bacteriostatic effects on several *Xf* strains representing different subspecies. High TTs (42, 44, 45, 46, 47, 48 and 50°C) were applied for short exposure periods (20 and 60 minutes). Subzero temperatures (-2 and -8°C) were tested for 20 and 60 min, and -15 and -20°C for longer exposures of 48, 96 and 168 h. Bacterial survival was assessed by monitoring growth in liquid culture, colony-forming unit (CFU) counts, biofilm formation and the quantification of viable cells using a viability-qPCR assay. Treatments above 42°C had variable effects under short exposure times. At 42 and 44°C, only approximately a 1-log reduction in CFU counts was achieved across most strains. In contrast, a significant detrimental effect on growth and biofilm formation was observed in most strains from 45°C onwards (growth reduction exceeding 90%), while treatments above 47°C completely inhibited both growth and biofilm formation. Interestingly, the CoDiRo (*Xf* subsp. *pauca*) strain exhibited greater tolerance to high temperatures. In general, cell viability decreased with increasing temperature and exposure time. A marked reduction in the percentage of viable cells was observed, with from 0.99 to 7.38% survival rates after 60 min of exposure. However, a small fraction of viable cells persisted even under TTs for which no growth or biofilm development was observed. Exposure to -2 and -8°C for 20 and 60 min led to a marked reduction in cell viability (55-79%), although a proportion of viable cells remained capable of recovering growth and forming biofilm after incubation at 28°C. In contrast, longer low temperature exposures at -15 and -20°C impaired growth after 96 h of treatment. These findings contribute to improving risk assessment models and support the development and optimization of TTs in nurseries as a complementary phytosanitary measure to ensure the production and movement of *Xf*-free plant propagation material.

P64. Mitigation of Olive Quick Decline Syndrome Through Endotherapy with Pomegranate Peel Extract

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The present research investigates the antibacterial efficacy of a pomegranate peel extract in controlling the development of Olive Quick Decline Syndrome (OQDS), a severe disease caused by *Xylella fastidiosa* subsp. *pauca* strain De Donno. This xylem-limited, systemic bacterium colonizes and obstructs the water-conducting vessels of olive trees, progressively impairing xylem sap flow and ultimately leading to canopy desiccation and plant decline. OQDS has become one of the most destructive diseases affecting olive cultivation in southern Italy, prompting the need for innovative, sustainable, and environmentally compatible control strategies. Over a six-year period (2016–2021), olive trees naturally infected by *X. fastidiosa* subsp. *pauca* were treated with a hydroalcoholic extract obtained from *Punica granatum* fruit peel. High-Performance Liquid Chromatography–Diode Array Detection–Electro-Spray Ionization Mass Spectrometry (HPLC-DAD-ESI/MS) analysis was performed to identify punicalagins and ellagic acid, the main bioactive compounds known for their antifungal and antibacterial properties. The field trial was conducted on a commercial farm in Trepuzzi (Lecce), located in the Salento area of southern Italy. Both in vitro and in vivo assays demonstrated the effectiveness of the extract. Treated olive trees exhibited a lower severity of canopy symptoms compared with untreated controls. Moreover, molecular analyses assessing the presence of *X. fastidiosa* confirmed the efficacy of the Pomegranate Peel Aqueous + Ethanol Extract (PPAEE) in reducing bacterial detection in treated plants.

P65. Oxylipins in *Xylella fastidiosa*–plant interaction: insights from bacterial and plant lipid signaling

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Lipids play a key role in regulating the ability of *Xylella fastidiosa* to switch between planktonic growth, twitching motility, and biofilm formation. These processes are crucial for host colonization and symptom development. In addition to diffusible signaling factors (DSFs), such as cis-2-unsaturated fatty acids, which have been extensively described, oxylipins (oxidized unsaturated fatty acids) are also emerging as important signals. Here, we investigated the role of oxylipin-mediated signaling in *X. fastidiosa* infection process by combining two complementary approaches addressing both bacterial and plant lipid pathways. First, we explored the contribution of the non-fimbrial adhesin XadA2, a trimeric autotransporter involved in surface attachment during early stages of biofilm formation. Deletion of the XadA2 gene in two different *X. fastidiosa* strains resulted in a strong reduction in twitching motility and biofilm formation. Lipidomic analyses revealed that the mutant accumulated the oxylipin precursor 10-HpOME, while showing reduced levels of 7,10-DiHOME, an oleic acid-derived oxylipin associated with biofilm development, suggesting that XadA2 is required for the signaling pathway leading to its synthesis. To investigate the contribution of plant lipid metabolism to this regulatory network, we analyzed the interaction between X. fastidiosa, and Arabidopsis thaliana plants impaired in the AtLOX2 gene, encoding a 13-lipoxygenase involved in plant 13-oxylipin biosynthesis. Reduction of 13-oxylipins in the lox2 mutant was associated with increased bacterial colonization and spread within plant tissues, indicating that plant-derived oxylipins contribute to limiting pathogen progression. Overall, our findings highlight the role of oxylipin-mediated signaling in shaping *X. fastidiosa* behavior and host colonization. These insights open new perspectives for the development of innovative strategies to control diseases caused by this devastating pathogen.

P66. Application of Pomegranate Peel Extract-based formulations to control *Xylella fastidiosa* infection: original evidences from root and endotherapy approaches

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Effective field management of *Xylella fastidiosa* subsp. *pauca* (*Xfp*) demands innovative and eco-friendly strategies. Pomegranate peel extract (PGPE), a bioactive byproduct of agricultural waste, represents a promising candidate given its well-known antimicrobial properties. To optimize its efficacy, this research evaluates PGPE through two targeted delivery systems: cellulose nanocrystals-amended pellets for root application and a thermoreversible gel for endotherapy. Initial results confirmed the PGPE in vitro inhibition of *Xfp* growth at concentrations as low as 0.5% w/v, supporting subsequent controlled environment trials on 3-years old olive plants and one year old cuttings of cultivars 'Leccino' and 'Ogliarola'. These trials monitored physiological parameters such as the Nitrogen Balance Index (NBI), root-to-plant weight ratio, and soil pH, alongside molecular and chemical profiling where gene expression related to photosynthesis, defense, oxidative stress, and hormone pathways was analyzed and complemented by sap metabolite profiling via UHPLC/HRMS. *In planta*, PGPE treatments demonstrated significant biostimulant effects and activated resistance-related genes, particularly 30 days post-treatment, with transcriptional and metabolic responses varying significantly between 'Leccino' and 'Ogliarola'. To validate these findings, ongoing field trials are being conducted across three naturally infected olive groves in Apulia (cultivar Leccino, Frantoio, and Ogliarola) where *Xfp*-positive and *Xfp*-negative plants have been treated with pellet and endotherapy gel and further monitored considering pathogen quantification (asessed by real-time qPCR). Under field conditions, NBI, chlorophyll, flavonoids, and anthocyanins are assessed using a non-destructive fluorescence-based approach, while leaf composition is analyzed through untargeted metabolomics. In addition, metabarcoding is planned to evaluate the impact of pellet application on soil microbial diversity. These comprehensive efforts, alongside preliminary data from tobacco pathosystem, highlight the potential of site-specific PGPE delivery to mitigate disease progression in *Xfp*-impacted regions.

P67. Update on *in planta* control and other risk reduction options for *Xylella fastidiosa*

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This scientific opinion updates previous EFSA Scientific Opinions on in planta control measures and other risk reduction options for *Xylella fastidiosa*. The potential in planta control measures targeting *X. fastidiosa* were identified through a systematic literature search, then described and critically appraised. This review addresses chemical and biological control treatments applied to living plants (model or crop plants), tested under controlled conditions and in field-scale experiments. *In vitro* studies evaluating antibacterial substances, and other disease management options, including agronomic practices such as weed management and pruning were also considered. Resistant or tolerant varieties, direct vector control and the use of antibiotics were excluded from the scope of this opinion. Treatment efficacy was assessed based on its effect on reduction of disease symptoms and bacterial populations. The evaluated treatments included bacterial isolates (e.g., *Paraburkholderia phytofirmans*), antimicrobial peptides, bacteriocins, fosetyl-Al nanocrystals, N-acetylcysteine, menadione, benzethonium chloride, zinc oxide and other mineral-based formulations, and plant-derived extracts. Although some treatments reduced disease symptoms under controlled and field conditions, these effects were rarely associated with a substantial reduction in bacterial populations in plants. The Panel confirms its previous conclusion that no in planta control measure is currently available to eliminate the bacterium from infected plants under open-field conditions in the EU.

P68. Microbial control of Xylella fastidiosa through a novel antagonistic protein from the plant phyllosphere

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(See thematic session 2.7c)

P69. Evaluation of Phenolic Extracts from *Olea europaea L.* via Endotherapy for the Management of *Xylella fastidiosa* in Olive Orchards

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Xylella fastidiosa is a xylem-limited bacterium responsible for Olive Quick Decline Syndrome (OQDS), causing severe vascular occlusion and plant death. This study investigates the efficacy of natural phenolic compounds, sourced from Coratina cultivar leaves, as a sustainable tool to mitigate symptoms and support growth in infected trees. The trials are conducted in Mesagne (Apulia, Italy), an area under high infection pressure, on three cultivars: Cellina di Nardò, Picholine, and Cima di Melfi. The experimental design involves the administration of phenolic extracts via endotherapy, tested alone, in combination with carriers, or as coatings for silver nanoparticles. A key component of the study is the characterization of the metabolomic profile of these extracts to correlate specific secondary metabolites with the observed biostimulant and antimicrobial effects. Plant response is monitored through longitudinal phenotypic assessments, measuring vegetative growth, canopy density, and symptom progression. These data aim to validate the use of olive-derived bioactive compounds as a core component of integrated, sustainable strategies for coexistence with *X. fastidiosa* in Mediterranean olive growing.

This research is supported by funds from the project: “Fenotipizzazione di genotipi di olivo resistenti a *Xylella fastidiosa* e messa a punto di un modello di gestione agronomica ad elevata sostenibilità ‘GENFORAGRIS’ (DM n. 664538 del 28/12/2022).

P70. Analytical Performance of a Novel Lyophilized Turnback LAMP (TLAMP) Assay for Rapid Detection of *Xylella fastidiosa*

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Xylella fastidiosa is a major quarantine plant pathogen threatening several economically important crops, requiring rapid and reliable diagnostic tools for phytosanitary surveillance. This study describes the development and analytical validation of a novel lyophilized Turnback Loop-Mediated Isothermal Amplification (TLAMP) assay for rapid detection of *X. fastidiosa*. To our knowledge, this represents the first TLAMP-based assay reported for this pathogen. Analytical validation included assessment of limit of detection (LOD), analytical sensitivity, specificity, repeatability, and robustness using serial dilution panels and four NCBI-registered *X. fastidiosa* isolates. The assay demonstrated stable and highly sensitive detection across all isolates and consistent performance during inter-laboratory evaluations. Comparative analyses using the DOSAbio *Xylella fastidiosa* qPCR Detection Kit and real time LAMP assay on samples derived from olive and grapevine hosts showed comparable diagnostic results. The lyophilized format further enables rapid, sensitive, and practical application under both laboratory and field conditions for phytosanitary diagnostics and surveillance.

P71. Ecophysiological characterization of olive old cultivars and new genotypes in Salento

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Olive plants are strongly affected by *Xylella fastidiosa* since 2008 in Salento, a land located in the South-Eastern Italy. The Mediterranean region is among the areas most strongly affected by climate change at a global level. As the xylem hydraulic conductance is affected by *X. fastidiosa* as well as by the climate stress induced in particular by the rising vapor pressure deficit (VPD), a possible interaction of biotic/abiotic stress can be considered as the cause of the Olive Quick Decline Syndrome (OQDS) occurrence. Physiological parameters were detected in centuries-old plants of Ogliarola salentina, an old traditional cultivar, located in the plane of Ostuni, and in 19 8-years old new genotypes coming from controlled crosses among different parents ('FS17', 'Leccino', 'Kalamata' and 'Dolce Agogia') located in an experimental orchard located in Monteroni (LE): leaf gas exchanges (stomatal conductance, transpiration, net CO2 assimilation and substomatal CO2 concentration), stem water potential (SWP), relative water content (RWC) and water use efficiency (iWUE) were analysed in 2024 and 2025 during spring, summer and autumn . Weather data (temperature, relative humidity and VPD) were also detected in the Monteroni collection field. The possible role of soil irrigation and foliar fertilisation and irrigation were studied in century-old plants, in order to evaluate the actual usefulness of water delivery during summer, when evapotranspirative demand is highest, and the possible role of foliar irrigation as a form of microclimate control to reduce the stress induced by high temperature and VPD. A significant role of foliar irrigation was found improving physiological status, and a role of foliar fertilisation inducing higher stomatal conductance and transpiration and lower stem water potential of century-old plants. Significant differences were also found among new genotypes as useful tools contributing to the choice factors of new genotypes suitable for the Salento environment.

P72. Tracing the origin of the new *Xylella fastidiosa* subsp. *fastidiosa* outbreak in Extremadura, Spain, through comparative genomic analysis and phage typing

Morán F., Selles C., Barbé S., Navarro I., Monterde A., Marco-Noales E.[

(See thematic session 3.7a)

P73. Hfq-dependent small RNAs mediating environmental sensing in Xylella fastidiosa support lifestyle transitions and virulence.

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(See thematic session 3.7e)

P74. First Draft Genome Sequence of *Xylella fastidiosa* from Citrus in the Amazon, Brazil

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Xylella fastidiosa is a xylem-limited bacterial pathogen of major global phytosanitary importance, affecting economically relevant crops such as citrus, olive, grapevine, and coffee. The Amazon region harbors one of the highest levels of plant biodiversity worldwide, and the occurrence of a broad host-range pathogen such as *X. fastidiosa* in this ecosystem raises important questions regarding host availability, adaptation, and epidemiological dynamics in tropical environments. In the Brazilian Amazon, its presence has previously been inferred solely through symptom observation and PCR detection, with no genomic data available from regional isolates. Here, we report the first draft genome sequence of a *X. fastidiosa* isolate obtained from symptomatic sweet orange (Citrus sinensis) collected in Rio Preto da Eva, Amazonas, Brazil. Additionally, three rRNA genes, 47 tRNAs, one tmRNA, and a complete ribosomal operon (16S–23S–5S) were identified. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit and sequenced using Oxford Nanopore technology with the Rapid Barcoding Kit V14 (barcode RB48). De novo genome assembly using Flye v2.9.6 generated a high-quality draft genome comprising 38 contigs totaling 2,876,366 bp, with an N50 of 232,727 bp, a GC content of 52.76%, and an average sequencing coverage of 53×. Genome quality assessment using CheckM indicated 99.64% completeness and 0.18% contamination, supporting the high quality of the assembly. Genome annotation performed with Prokka v1.15.6 identified 2,916 coding sequences (CDS), including 1,612 hypothetical proteins and 1,304 genes with predicted functions. Functional annotation using eggNOG-mapper (Galaxy platform) revealed genes associated with type II, IV, and V secretion systems, type IV pili components, hemagglutinin-related proteins, and elements of the rpf quorum-sensing regulatory system (including rpfC and rpfG), consistent with conserved mechanisms involved in biofilm formation and virulence in *X. fastidiosa*. This genome represents the first genomic reference of *X. fastidiosa* from the Brazilian Amazon and provides a valuable resource for future comparative, evolutionary, and epidemiological studies in biodiversity-rich tropical agroecosystems.

P75. Seasonal lipidomic profiling for the identification of biomarkers associated with olive quick decline syndrome

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Lipid signaling molecules play a central role in plant responses to biotic stress and are increasingly recognized as key mediators of host–pathogen interactions. In the olive–*Xylella fastidiosa* subsp. *pauca* (*Xfp*) pathosystem, free fatty acids and oxylipins have been identified as molecules capable of modulating *Xfp* behaviour, promoting or inhibiting the transition between planktonic growth and biofilm formation, a key step for the colonization of xylem vessels. Several of these lipid compounds have also been previously reported as potential discriminants between OQDS-positive and OQDS-negative olive trees. This study investigates the lipidomic alterations associated with *Xfp* infection in olive trees by monitoring lipid and hormonal profiles in relation to seasonal fluctuations, cultivar differences, and symptom severity. The aim is to evaluate how these factors influence lipid composition and to identify stable, reliable metabolic biomarkers of infection. Ultimately, the objective is to determine lipid signatures potentially associated with olive quick decline syndrome (OQDS) that remain consistent regardless of seasonality, symptom intensity, or the bacterial load present in the plant. Sampling was carried out over a one-year period – July 2024, November 2024, February 2025 and May 2025 – on olive trees belonging to two cultivars typical of the Salento area, Ogliarola Salentina and Cellina di Nardò. Samples were collected from five different fields and classified according to different symptom severity stages. The presence of *Xfp* in plant tissues was confirmed by real-time PCR analysis. Metabolite characterization was carried out using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC–MS/MS) through a targeted approach aimed at quantifying specific lipid classes and plant hormones. The analytical method was further optimized by implementing dedicated acquisition time windows for each analyte class to improve sensitivity, selectivity, and robustness when analyzing complex plant matrices. Preliminary results highlight significant variations in the lipid profile in relation to symptom severity. Overall, this study aims to provide new insights into seasonal lipidomic dynamics associated with *Xfp* infection and to identify potential metabolic biomarkers associated with the disease for evaluate the potential of lipidomic approaches as complementary tools to traditional molecular methods.

P76. Preliminary transcriptomic profiles of three almond cultivars in response to the infection of different *Xylella fastidiosa* subspecies

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(See thematic session 3.7b)

P77. Epidemic of olive quick decline syndrome in Apulia: monitoring the changing scenario

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In southern Apulia (Italy), a decade after the initial outbreak, the rapid spread and devastating impact of *Xylella fastidiosa* (*Xf*) subsp. *pauca* ST53 in olives have led to a self-limiting reduction in inoculum pressure, due to the decline of the infected olives, the main host species in the infection's epidemiology. This changing scenario probably explains the reduced severity of desiccation symptoms observed in the new growth of surviving, yet diseased, olive trees. To gather experimental data supporting this evidence, we have monitored 16 olive groves, 8 located in the area with long-established infection (Salento peninsula - SLP) and 8 in the northern border of the epidemic area (Brindisi and Taranto provinces - BTP). In both locations, five century-old olive orchards and three newly established super-high-density (SHD) orchards with resistant cultivars were monitored for vector infectivity. Briefly, from 2022 to 2025 starting in June till October these olive groves were monthly monitored, for the adult population density of *P. spumarius* and the occurrence of *Xf*-positive specimens estimated by qPCR. In parallel, 10 pathogen-free potted olive plants were placed in olive grove and, at the end of each year, transferred in screenhouse and tested in the next summer season. During the first year, the percentage of insects testing positive for *Xf* was 19.8% in the SLP area and 48.5% in the BTP area. Thereafter, a progressive reduction in the proportion of *Xf*-positive specimens was observed in both areas, reaching 5.0% in SLP and 20.0% in the BTP. A similar trend was recorded on the potted plants exposed to the natural infection in both areas. Overall, the data indicate a progressive reduction in the inoculum pressure in both study areas. The decrease of *Xf*-positive *P. spumarius* in the long-established outbreak is consistent with the reduction of infected olive tree, leading to reduced pathogen reservoirs and reinfection rates. Whereas in recently affected areas this trend is likely driven by the increasing disease impact, which limits suitable feeding sites for vectors and, consequently, pathogen acquisition and spread. The reduced incidence of infective vector specimens, correlates with a reduced pressure of inoculum (reduced transmission events), likely explaining the observed amelioration in the condition of diseased trees following the peak of the epidemic.

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P78. Optimising Juvenile Spittlebug Monitoring Through a Stratified Two-Step Collection Method

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The census of spittlebug vectors of *Xylella fastidiosa* is essential for insect ecological-demographic studies and for the success of Integrated Pest Management (IPM). Our proposal, aka AcquaSamPling, uses a standardised two-step collection method of juveniles on their host plants by an (1) active water rinsing and sifting followed by a (2) passive second scrutiny to detect insects that were eventually missing. Sampling employed a destructive quadrat (400 cm^2), with all plants collected at soil level and preserved in labelled plastic bags for laboratory scrutiny. An optimal stratified design accounted for vegetation structure, phenology and host plant preference, allocating samples proportionally to expected variability. The objectives were to determine whether the second step significantly increased counts, identify threshold conditions for its added value and assess consistency across plant associations, soil use, IPM, and sampling times. Descriptive measures (mean, standard deviation, coefficient of variation) and inferential analyses (Bayesian ANOVA and non-parametric tests) were applied. Results showed that the two-step method significantly increased detection ($p < 0.05$), with the second step, on average, adding 19.2% more individuals. Combined counts had a lower coefficient of variation (129.4%) than either step alone. This reduction in variability indicates that repeated examinations decrease detection error and stabilise abundance estimates. The benefit was greatest when first-step counts were low (<10), especially with 0–5 individuals. This pattern is consistent with the patchy distribution and concealment behaviour of juvenile spittlebugs within foam masses and plant structures, which can hinder detection during a single inspection. In about 15% of samples where the first step detected none, the second step identified at least one insect, but if first-step counts exceeded 30 individuals, the additional step was minimal. Overall, the two-step method enhances detection reliability and reduces sampling variability, particularly in low-density or newly surveyed populations. The approach proved robust across ecological and agronomic conditions, providing a practical framework for optimising monitoring effort in juvenile spittlebug surveys and improving decision support for IPM. Finally, the combination of the two steps and the techniques employed makes data collection faster and more inexpensive than other proposals.

P79. Novel entomopathogenic fungi isolated from naturally infected populations of *Philaenus spumarius* as potential biological control agents

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The meadow spittlebug *Philaenus spumarius* (Hemiptera: Aphrophoridae) is a highly polyphagous xylem-feeding insect widely distributed across diverse habitats, including grasslands, meadows, and agroecosystems. This species represents a major agricultural concern due to its role as the primary vector of *Xylella fastidiosa*, a plant-pathogenic bacterium responsible for severe and currently incurable diseases affecting economically important crops such as olive, almond, and grapevine. During xylem feeding, *P. spumarius* efficiently transmits the pathogen, leading to symptoms including leaf scorching, reduced plant vigor, growth decline, and substantial yield losses. Current management approaches for *P. spumarius* rely mainly on mechanical suppression of nymphal stages, such as mowing or soil tillage, chemical control targeting adults, and the application of entomopathogenic fungi (EPF) as a sustainable alternative to synthetic insecticides. EPF naturally contribute to insect population regulation by infecting and killing their hosts, and in some cases are also known to alter host behavior. Several fungal species, including Beauveria bassiana and Metarhizium anisopliae, have already been investigated as potential biological control agents against *P. spumarius*. To identify additional EPF strains with activity against *P. spumarius*, we isolated fungi associated with natural infections of *P.* spumarius. Adults exhibiting advanced symptoms of fungal disease were collected in autumn from a population experiencing a widespread fungal outbreak. Fungal isolation was performed by culturing tissues dissected from freshly deceased adults on Potato Dextrose Agar (PDA) amended with lactic acid to suppress bacterial contamination. Following incubation, morphologically distinct colonies were selected for molecular identification. Genomic DNA was extracted and conserved ITS and TEF regions were amplified and sequenced to determine fungal identity. This approach led to the identification of five putative EPF species: *Fusarium proliferatum*, *Cladosporium tenuissimum*, *Aspergillus oryzae*, *Aspergillus ochraceus* and *Penicillium olsonii*. Two out of five fungal species tested in laboratory bioassays showed high pathogenicity against adults of *P. spumarius* and are being evaluated as potential biocontrol agents. This study was conducted within the framework of the REACH-XY project.

P80. Genomic characterization of Wolbachia suggests its functional integration in a multipartite bacterial symbiosis in *Philaenus spumarius*

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Philaenus spumarius hosts a complex bacterial community that includes two obligate nutritional endosymbionts and facultative endosymbionts with unknown functions. Wolbachia bacteria establish relationships with many insects by acting as reproductive manipulators or as mutualistic symbionts. The role played by Wolbachia in *P. spumarius* is unknown. Our surveys along the Italian peninsula indicate that the prevalence of Wolbachia infection in *P. spumarius* populations is not influenced by host genotype, but is strongly associated with environmental conditions, with higher infection rates in colder environments and no infection in southern Italy. We characterized the assembled genome of the most widespread Wolbachia strain (ST539) in Italian *P. spumarius* populations and studied the potential functional role of ST539 within a symbiotic consortium including the obligate nutritional symbionts Candidatus Karelsulcia muelleri and a Sodalis-like bacterium. The 1.89 Mb circular genome exhibits canonical Wolbachia features, including low GC content, abundant mobile genetic elements, and multiple intact WO prophages, indicative of a dynamic genome shaped by recombination and horizontal gene transfer. The presence of 45 ankyrin repeat–containing proteins suggests extensive potential for protein–protein interactions within the host and among symbionts. Metabolic pathways reconstruction reveals that Wolbachia retains the genetic capacity for riboflavin biosynthesis and encodes most components of folate-associated metabolism. In contrast, Karesulcia, specialized in essential amino acid provisioning, lacks genes involved in these vitamin pathways. Comparative analyses show that Wolbachia and the Sodalis-like symbiont share some vitamin-related and core metabolic genes but differ in pathway completeness, indicating complementary rather than redundant metabolic roles. Phylogenetic analyses reveal that homologs of the cytoplasmic incompatibility (CI) proteins cifA and cifB, found in ST539 cluster within CI-expressing Wolbachia clades. Although the phenotypic effects of cif genes of ST539 remain unresolved, their presence indicates retention of CI-related genetic determinants. Our results support a model in which Wolbachia acts as a metabolically competent and ecologically flexible symbiont, contributing to a division of metabolic labor within a multipartite mutualistic symbiosis and extending its ecological role beyond CI. This study is part of the REACH-XY project.

P81. *Metarhizium brunneum* direct exposure reduces survival and disrupts feeding behaviour in *Philaenus spumarius*

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(See thematic session 4.8b)

P82. Simple and Rapid Detection of Salivary Sheaths at *Philaenus spumarius* feeding Points

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Philaenus spumarius L., 1758 (Ps), is a vector of *Xylella fastidiosa* (*Xf*), posing a major threat to agriculture worldwide. During feeding, *Ps* inserts its piercing-sucking mouthparts into the apoplast and secretes salivary sheaths that seal the wound, thereby reducing the risk of embolism. The sheaths remain in plant tissues after feeding and can be used to investigate Ps feeding behaviour and estimate potential *Xf* transmission events. Feeding acts can be classified as pre-infective (before *Xf* acquisition), infective (during *Xf* transmission), and post-infective (after a previous successful transmission event). The classification provides a useful framework for interpreting epidemiological risk, vector activity, and transmission pressure. In this study, we propose a rapid method to detect and localise *Ps* salivary sheaths on host and food plants. Stem section from herbaceous plants hosting juvenile stage, as well as olive twigs and leaves, which are among the food sources of adult *Ps*, were manually sliced and preserved in 75% ethanol (v/v). Lab experiments show that, apart from the slices' direct scrutiny, stained slide-mounted 1 mm-thick slices suggest that acid fuchsin, chlorazol black, and phloroglucinol stained the salivary sheaths, but often reduced contrast, making the slices and the salivary sheaths less readable. Nevertheless, Essig's fluid and benzyl alcohol diaphanised the slices, slightly shifting their colour. Generally, staining is time-consuming and hazardous due to dye carcinogenicity; thus, we concluded that observing slices in 75% EtOH in water is effective to spot *Ps* salivary sheaths. These techniques allow us to locate xylem-sap feedings in about 15 cm of olive twig, including its leaves, per working hour. We presume these tools will help us quantify the potential number of pathogen transmission events or spot vector preferences for olive clones, thereby supporting to incorporate resistance in the development of robust, effective plant pathogen management strategies.

P83. Environmental factors override host genotype in shaping Wolbachia endosymbiont prevalence in *Philaenus spumarius*

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Philaenus spumarius is a key vector of the invasive plant pathogen *Xylella fastidiosa* in Europe. Wolbachia are bacterial symbionts of many insects, able to spread within host populations as reproductive manipulators or as mutualistic symbionts. *P. spumarius* hosts Wolbachia but the type of symbiotic interaction and the factors shaping its distribution within host populations remain unclear. Phylogeographic analyses have identified three main mitochondrial lineages in *P. spumarius*: the north-eastern (NE), the eastern-Mediterranean (East-Med) and the western-Mediterranean (West-Med) lineages, which show distinct geographic distributions. Our previous surveys along the Italian peninsula revealed the absence of Wolbachia in *P. spumarius* populations from southern Italy (East-Med genotype), while the prevalence of infection increased along an altitudinal gradient in populations from northern Italy (West-Med and NE genotypes), suggesting a role of environmental conditions, although an effect of the host genotype could not be excluded. In this study, conducted within the REACH-XY project, we expanded the geographic and altitudinal sampling across Italy to reassess Wolbachia infection patterns in relation to mitochondrial lineage and environmental variables. Populations from southern, central, and northern Italy were analyzed using COI gene sequencing, diagnostic PCR for Wolbachia detection, and MLST for characterization of the symbiont at the strain level. Wolbachia was consistently absent in populations from southern Italy, even at high altitudes. In northern Italy, high infection rates were detected in NE and West-Med populations sampled at high altitudes (1200–2000 m a.s.l.), whereas West-Med populations at low altitudes showed variable infection prevalence. In central Italy, Wolbachia infection rate was very high in East-Med populations sampled at high altitudes (1000–1500 m a.s.l.). These results rule out host mitochondrial genotype as a major determinant of Wolbachia distribution and point to environmental factors as the primary drivers. Cold and snowy conditions, such as those found at high altitudes, combined with a shorter insect's ovarian parapause, appear to favor Wolbachia persistence, whereas prolonged heat and drought typical of the Mediterranean climate in southern Italy may limit infection. This knowledge could help clarifying the role played by Wolbachia in *P. spumarius* and the exploitation of Wolbachia in the sustainable control of the vector.

P84. Transmission efficiency of *Xylella fastidiosa* subspecies by *Philaenus spumarius*: a comparative study of ST53 and ST81

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Xylella fastidiosa (*Xf*) is one of the most damaging plant pathogens, infecting a wide range of economically important crops such as olive, grapevine, almond, and citrus. Since its introduction into the Mediterranean basin in 2013, *X. fastidiosa* has caused severe damage to agriculture and to the landscape in the region. The bacterium was first detected in Apulia (southern Italy), where it caused Olive Quick Decline Syndrome (OQDS), devastating billions of olive trees. In the Balearic Islands, several subspecies are already established across multiple host plants, severely affecting almond production and grapevines, and to a lesser extent olive orchards. In 2024, the highly virulent strain *X. fastidiosa* subsp. *pauca* ST53, responsible for the Italian outbreak, was detected in Mallorca, raising serious concerns about the future of olive production on the island and to the potential extension of this aggressive variant to other areas. The bacterium is transmitted by xylem-feeding insects, being the meadow spittlebug *Philaenus spumarius* the main vector in Europe. In this study, we conducted an open-field transmission experiment within a quarantine area where *X. fastidiosa* subsp. *pauca* ST53 had been detected, aiming to investigate the transmission dynamics of *P. spumarius* and its interactions with two bacterial strains: the recently introduced *X. fastidiosa* subsp. *pauca* ST53 and the already established *X. fastidiosa* subsp. *multiplex* ST81. Our results demonstrate the high pathogenic potential of *X. fastidiosa* subsp. *pauca* ST53, confirming the significant risk this strain poses to European agriculture. We found that *P. spumarius* acquires *X. fastidiosa* subsp. *pauca* ST53 at significantly higher rates than *X. fastidiosa* subsp. *multiplex* ST81 when both strains occur in sympatry, suggesting a strong potential for its spread. Furthermore, the vector was able to co-acquire and co-inoculate both subspecies into host plants, highlighting the possibility of mixed infections in natural systems. These findings emphasize the importance of monitoring vector–pathogen interactions and assessing the potential for recombination between strains, which could influence pathogen virulence, host range, and future disease dynamics.

P85. Visual and olfactory drivers of intervarietal discrimination in the meadow spittlebug *Philaenus spumarius*

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While previous research on *Philaenus spumarius* host-seeking has focused primarily on olfactory cues, the vector's visual ecology remains a "black box" in our understanding of *Xylella fastidiosa* epidemiology. Our prior characterization of the spittlebug's visual system revealed a multifaceted sensory apparatus. X-ray micro-CT and pseudopupil tracking identified a moderate frontal acute zone, with interommatidial angles ranging from 3 to 6 degrees, while single-cell electrophysiology confirmed a trichromatic system (UV, blue, and green) with high polarization sensitivity in the UV and blue channels. Beyond this physiological evidence, observed behavioral preferences for linearly polarized light suggest that polarization vision may be a crucial driver for host detection at long-to-medium ranges. The current study investigates the functional role of these optical signatures in facilitating discrimination among *Olea europaea* varieties. By integrating polarization-sensitive imaging with high-resolution spectrophotometry, we quantified the leaf reflectance profiles of diverse olive varieties. This allowed us to select cultivar pairs based on a four-way matrix of visual contrast consisting of pairs: i) with divergent color and similar polarization; ii) pairs with divergent polarization and similar color; iii) pairs with divergence in both channels; iv) and pairs with visual parity in both cues. To determine the hierarchy of these sensory inputs, we tested these cultivars in specialized dual-choice bioassays that isolate visual stimuli, effectively decoupling the influence of spectral reflectance (brightness and hue) from the degree of linear polarization. Parallel to these visual assays, the same cultivar pairings were evaluated in olfactometer trials to determine whether the volatiles contribute to host location and inter-varietal discrimination. This comparative framework aims to define the sensitivity of the vector's visual system and its role in host selection. By identifying specific optical profiles that reduce vector attraction, this research provides a technical basis for selecting less attractive cultivars as a sustainable tool for curbing vector activity and mitigating *X. fastidiosa* spread.

P86. *Draeculacephala robinsoni*, a potential vector of *Xylella fastidiosa* in Europe: Distribution and population dynamics across the Iberian Peninsula

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(See thematic session 4.8a)

P87. Assessment of insect vector competence for the acquisition and transmission of *Xylella fastidiosa* to agricultural and forest plant species in Switzerland

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(See thematic session 4.8d)

P88. Olfactory responses of *Philaenus spumarius* to botanical products: an innovative behavioural approach

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The management of insect vectors of plant pathogens remains a major challenge for sustainable agriculture, as vector behaviour strongly influences disease transmission dynamics. The meadow spittlebug *Philaenus spumarius* (*L.*) (Hemiptera: Aphrophoridae), the main vector of *Xylella fastidiosa* subsp. *pauca* in the Mediterranean region represents a key target for behaviour-based control strategies. In this study, we evaluated the potential of a novel open-arena system coupled with automated video tracking and electrophysiological techniques to characterize olfactory-mediated behavioural responses of *P. spumarius* to *Pimpinella anisum L.* essential oil (EO). Behavioural assays revealed a concentration- and time-dependent response, with 3% anise EO producing the strongest deterrent effect on green beans. This concentration was therefore selected for fine-scale automated video-tracking assays. Using a 3D-printed open arena combined with EthoVision® XT video tracking, we evaluated first choice, visit frequency, cumulative duration, and locomotor speed in adult males and females. Both sexes showed a preference for control substrates, with reduced visitation and residence time in EO-treated zones, and increased walking speed, indicating active avoidance. Females exhibited stronger avoidance responses than males. Electrophysiological analyses supported the behavioural results. GC-MS-EAD recordings identified five antennal active compounds in the EO, with (E)-anethole eliciting the strongest responses. EAG experiments revealed increasing antennal sensitivity with EO concentration and higher female sensitivity at intermediate doses. By integrating electrophysiology with automated insect tracking, this study links olfactory perception with functional behavioural outcomes, and highlights the potential of advanced behavioural tools to detect subtle, sublethal effects of botanical products. These findings support the potential use of anise EO in sustainable management of *X. fastidiosa* vectors, with special reference to behaviour-based approaches.

P89. Where are the vectors? Searching for xylem feeders associated with *Xylella fastidiosa* subsp. *multiplex* host plants in Central Italy

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In 2018, *Xylella fastidiosa* subsp. *multiplex* ST87 (*Xfm*) was first detected in Monte Argentario, Central Italy. This area is characterized by a typical Mediterranean vegetation, and plant species like *Spartium junceum* (*Sj*), *Rhamnus alaternus* (*Ra*) and *Calicotome villosa* (*Cv*) are frequently detected as *Xfm*-positive during surveillance programs. Among potential vectors, *Philaenus spumarius* (*Ps*) and *Neophilaenus campestris* (*Nc*) are the only species tested positive for *Xfm* so far. Moreover, their ability to transmit *Xfm* to *Ra* was assessed under laboratory conditions. Although spittlebugs, especially *Ps*, were recognized as the European *X. fastidiosa* vectors, field surveys carried out in Monte Argentario did not clarify their relationship with *Xfm* host plants. Indeed, *Ps* and *Nc* were rarely observed on *Sj*, *Ra*, and *Cv*, complicating the understanding of *Xfm* epidemiology in Central Italy. In 2024-2025 a targeted field sampling was conducted in six demarcated areas of Monte Argentario to search for spittlebugs and other xylem feeders on *Sj*, *Ra* and *Cv*. The sampling was performed monthly from May to December, at different times of the day. Insects were collected by hand on plants after visual inspection or by sweeping net or beating net, then preserved in 96% ethanol. After taxonomic identification, specimens were analyzed to detect *Xfm*. Xylem feeders collected on target plants included *Ps*, *Nc*, *Cercopis vulnerata*, and C. sanguinolenta. All species were found on *Ra*, whereas *Ps* was also collected on *Sj* and *Cv*, highlighting its broader host range compared to the other observed xylem feeders. However, spittlebugs and froghoppers were only sporadically captured in comparison with other Auchenorrhyncha species. Indeed, Issidae planthoppers are the most abundant taxon found on all plant species, across all sampling sites and throughout the sampling period. Thus, while phloem feeders occured consistently on *Xfm* host plants, xylem feeders were rarely found on them, confirming observations from previous surveys conducted in the same area. These results contrast with the general knowledge of *X. fastidiosa* pathosystems, in which vectors frequently exploit *Xf* host plants as food sources. Moreover, none of the analyzed specimens tested positive for *Xfm*. Overall, the results of this study reinforce previous observations and highlight the need for further research to clarify the role of Ps and other spittlebugs in the transmission of *Xfm* to plant species in Mediterranean biotopes.

P90. Haphazard effects of chemical control on the transmission of a vector-borne plant pathogen

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Vector-borne pathogens cause some of the most damaging plant diseases worldwide. Control strategies often rely on pesticides targeting insect vectors, yet their effectiveness in reducing pathogen spread is frequently inconsistent. Pesticide exposure may also alter vector behavior and physiology, with potential consequences for pathogen transmission dynamics, but these effects are rarely considered in integrated pest management strategies. Moreover, toxicological assessments usually focus only on insecticides targeting vectors, overlooking other pesticides, including fungicides, to which vectors are exposed and whose effects on pathogen transmission remain largely unexplored. Here we propose a framework to evaluate how pesticide exposure affects the transmission risk of vector-borne plant pathogens using a proxy of the basic reproduction number (R_0P). Based on a simplified Ross–Macdonald model, the metric integrates vector survival and the probabilities of pathogen acquisition and inoculation following topical or residual pesticide exposure. We applied this approach to the *Xylella fastidiosa* ST53–olive–*Philaenus spumarius* pathosystem, estimating R_0P values for major insecticides and fungicides used in olive canopies. Some of the pesticides tested altered pathogen transmission risk, either decreasing it (e.g., spinetoram and orange essential oil) or increasing it (e.g., copper-based fungicides). The model has limitations that restrict its predictive use. Survival data were obtained under artificial no-choice conditions and do not capture more complex responses such as pesticide-induced repellency. For instance, although acetamiprid caused complete mortality in our assays, it may act as a repellent under field conditions, potentially displacing vectors rather than killing them. The model also does not incorporate pesticide-induced changes in vector–host interactions, host preference, or longer-term effects on vector population dynamics. Nevertheless, our results show that pesticides—even those not targeting vectors—can influence the epidemiology of vector-borne plant pathogens, highlighting the need to consider sublethal pesticide effects when designing disease management strategies.

P91. *Zelus renardii*: a useful pest antagonist in orchards

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Philaenus spumarius is the key pest of olive groves in southern Italy due to its ability to acquire and transmit *Xylella fastidiosa*. To effectively counteract *Xylella* transmission, biological control actions need to be integrated into IPM strategies for olive orchards. However, the current arsenal of vector natural enemies is negligible. Therefore, we outline a biological control strategy based on the Nearctic predator *Zelus renardii* (Leafhopper Assassin Bug, LAB), which is well-established in many Mediterranean countries. We evaluated the feasibility of using *Z. renardii* to suppress *P. spumarius* populations and thereby control *X. fastidiosa* transmission. Predation tests, conducted on caged plants and on different orders of live prey with sizes ranging from 1/5 to 5/4 the size of the predator, confirm *Z. renardii*'s preference for free-living, highly mobile hemipterans. Simulations of mass release indicate that timely, planned releases can reduce the incidence of new infections to below 10% per year, supporting the inclusion of LAB in integrated defense programs. The predatory action of *Z. renardii* on other olive pests further supports its value as a multifunctional component of plant health management. From an eco-ethological point of view, analysis of the volatilome of *Z. renardii*'s Brindley glands revealed 2-methylpropanoic acid, 2-methylbutanoic acid, and 3-methyl-1-butanol, which can induce alarm responses in conspecifics and are potentially relevant to predatory efficiency in the field. However, we developed protocols for rearing the predator on artificial oligidic, meridic and holidic diets, allowing the completion of post-embryonic development for two successive generations. Prilling and vibration techniques enabled the production of sterile, long-term-storable microbeads and microcapsules, suitable for logistical management of releases. Comparative tests between wild and laboratory-reared LABs also revealed an initial predatory learning phase, followed by rapid behavioral convergence and increased effectiveness against *P. spumarius*. Overall, evidence supports *Z. renardii* as a promising biological control agent for vectors. Field validation will be the decisive step toward implementing a scalable, ecologically sustainable strategy that is consistent with the objectives of protecting olive production and the health of Mediterranean agroecosystems.

P92. The Meadow spittlebug postembryonic development and consequences

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The *Philaenus spumarius* exhibits a particular heterometabolic-hemimetabolous postembryonic development. At their first feeding, newborns begin to emit a mixture of liquid faeces and gland secretions, which bubbles into spittle-like, foamy masses. The spittle shelters the juveniles from severe environmental and biological hazards, creating a unique fluid, liquid, and atmospheric habitat on the host plants where juveniles thrive. Adults acquire and transmit *Xylella fastidiosa*. The lifestyle has consequences, the main one being that juveniles are aquatic and take oxygen directly from the liquid via the bubble interface. Juveniles do not exhibit trachea, move slowly and live confined in the spittle habitat on the host plant. Nevertheless, spittle protects juveniles from microorganisms, including bacteria and fungi, as well as antagonists. A real-time, third-generation Nanopore DNA sequencing protocol, along with an ad hoc bioinformatic workflow, using Kraken2 for taxonomic assessment against the RefSeq 2023.08 database, enabled the identification of microorganisms living in the spittle. The genera Pseudomonas, Microbacterium, Agrobacterium, and Stenotrophomonas appeared to be quite dominant, whereas other genera, such as Rhizobium and Brucella, were less abundant. An ESI-RPLC-MS/MS with an Orbitrap analyser, run on an aqueous extract in both ionisation modalities, showed few signals that were very difficult to interpret, since the exact composition varies with host plant species, sample, or preparation. Main components are water, proteins, and carbohydrates, with smaller amounts of fatty acids and inorganic species that act as natural surfactants. Peptide mass fingerprinting of tryptic digests by MALDI-MS analysis enabled the recognition of proteins such as Protein Wnt, Histone H2A, and Histone H3, all possibly involved in microbial management. Living in a minute, self-made fluid habitat has consequences: the spittle impedes *P. spumarius* colonisation of hot, dry habitats, restricting juveniles to early spring in Italy. Spotting or capturing juveniles is easier than catching adults and can be inexpensive using rinsing/sifting methods; monitoring will also be consistent. Juvenile control actions will be effective, set by conventional synthetic chemical formulations or sustainable, unconventional mortality factors. Finally, the bacteriostatic/bactericidal properties of spittle suggest new, unexplored options.

P93. Host plant selection and behavioural responses of *Philaenus spumarius* to herbaceous species

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Philaenus spumarius (L.) is the main vector of *Xylella fastidiosa* in Europe, and its host plant selection behaviour plays a key role in the epidemiology of this pathogen. Understanding how this insect responds to different host plants is essential for the development of effective management strategies. In this study, we evaluated the host plant preference of *P. spumarius* nymphs and adults in relation to four herbaceous species commonly found in Mediterranean olive groves (*Coleostephus myconis*, *Sonchus tenerrimus*, *Calendula arvensis* and *Chrysanthemum segetum*). Nymphal preference towards different herbaceous host plants was assessed under controlled conditions at different observation times (2, 12, 24 and 72 h). Adult behavioural responses to these plants, as well as to olive (Olea europaea), were evaluated using a multi-chamber olfactometer. In addition, the volatile organic compounds (VOCs) emitted by the host plants were characterised using HS-SPME/GC-MS to explore their potential role in insect attraction. Results showed that plant species significantly influenced host selection by *P. spumarius*. Nymphs initially preferred *C. myconis*, but over time shifted towards *S. tenerrimus*, which became the most selected plant after 72 hours. Adult insects exhibited a significant olfactory preference for *S. tenerrimus*, followed by *C. myconis*, while C. arvensis was the least attractive species. Volatile analysis revealed that although *S. tenerrimus* and *C. myconis* emitted fewer compounds, they shared dominant VOCs such as (Z)-3-hexen-1-ol, which may play an important role in mediating insect attraction. These findings highlight the importance of plant species and chemical cues in shaping the behavioural responses of *P. spumarius*, providing valuable insights for the development of targeted strategies to manage this vector and limit the spread of *X. fastidiosa*.

P94. Phenology and life cycle of *Philaenus spumarius* under semi-field conditions in northern Portugal

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Philaenus spumarius (L.) is the main vector *of Xylella fastidiosa* in Europe, and its life cycle plays a crucial role in the epidemiology and spread of this pathogen. Understanding its developmental dynamics under semi-natural conditions is essential to improve monitoring and management strategies. In this study, the biology and seasonal development of *P. spumarius* were investigated under semi-field conditions in Bragança (Portugal) over two consecutive years. Oviposition was confirmed in both years, although only a low number of egg masses were recorded. Despite this, nymph emergence occurred in all cages, indicating that oviposition took place even when eggs were not detected during inspections. Nymph emergence began in late February in 2024 and early March in 2025, occurring in a staggered sequence of instars. Early instars (N1–N2) dominated in March, followed by intermediate stages (N3–N4) in April and May, while fifth-instar nymphs (N5) were only recorded in 2025. By late May, nymphs were no longer detected. Adult emergence occurred from late April to late May in 2024 and from late May to early June in 2025. A total of 194 and 330 adults emerged in 2024 and 2025, respectively, with a slight male bias in both years. These results highlight the difficulty of detecting eggs under semi-natural conditions and confirm that *P. spumarius* exhibits a well-defined, temperature-driven seasonal development, with nymphal stages concentrated in early spring and adult emergence occurring in late spring to early summer.

P95. Uncovering viral partners: how the virome shapes fitness in *Philaenus spumarius*

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(See thematic session 4.8c)

P96. Landscape-scale population dynamics of *Philaenus spumarius* in Mediterranean agroecosystems

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Effective management of *Xylella fastidiosa* requires understanding the spatial and temporal dynamics of its vectors. However, the landscape-scale population dynamics of its main European vector, *Philaenus spumarius*, remain poorly understood, particularly during the dry Mediterranean summer. We investigated the spatio-temporal distribution of *P. spumarius* across five heterogeneous agricultural landscapes of 600 x 600 m in Apulia (southern Italy) over two consecutive years. Juvenile and adult populations were monitored in woody crops and unmanaged habitats (n = 168) to assess the effects of herbaceous vegetation, ground management, and seasonality on their abundance. In addition, 111 plots were sampled along five transects extending between major topographical depressions to test whether these depressions act as refugia during dry summer periods. In woody crops, nymph abundance was positively associated with vegetation structure (height and cover) and vigour and was markedly lower in recently managed plots. In unmanaged habitats, juvenile abundance was positively associated with plant species richness. Despite comparable nymph densities across landscapes in spring, adult populations declined sharply after emergence in most areas, with near-complete local disappearance during summer and widespread recolonization in autumn. Persistent summer populations occurred primarily in landscapes with a high proportion of unmanaged habitats and near topographical depressions. In autumn, vector abundance in woody crops was positively associated with herbaceous vegetation cover and was linked to nymph abundance in the following spring. Our results show that *P. spumarius* population dynamics are strongly context-dependent and shaped by local management, vegetation structure, and topographical conditions. These findings highlight the limitations of purely local control measures and underscore the need to incorporate landscape heterogeneity and seasonal connectivity into risk-based strategies for sustainable management of *X. fastidiosa*.

P97. This plant is taken: ant-associated cues affect spittlebug-plant interaction

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Non-consumptive effects (NCEs) are impacts predators exert on prey without directly killing or consuming them, typically through behavioral, physiological, morphological, or life-history changes triggered by the perception of predation risk. The mere perception of predators may therefore alter insect–plant interactions, potentially affecting the behavioral steps underlying pathogen transmission by insect vectors. Characterizing NCEs in insect vectors of plant pathogens could thus offer environmentally sustainable strategies to limit vector activity and mitigate pathogen spread. In this study, we investigated whether cues associated with the ant Crematogaster scutellaris influence host plant acceptance and settling behavior of the meadow spittlebug *Philaenus spumarius*, the main European vector of *Xylella fastidiosa* to olive. We characterized spittlebug responses to ant-associated cues by combining electropenetrography (EPG), used to evaluate probing behavior under restrained conditions, with video tracking using EthoVision XT to quantify activity and within-host plant preferences of freely moving insects. Treatments included playback of ant stridulation signals, ant chemical traces, their combination, and the presence of *C. scutellaris* individuals to infer the potential contribution of visual cues. Exposure to vibrational cues increased plant abandonment, altered within-plant behavior, and reduced xylem sap ingestion. In contrast, chemical cues affected earlier stages of host use, delaying stylet insertion and the onset of probing activities. The combination of vibrational and chemical cues did not produce additive effects, suggesting possible neural competition during cue perception. Automated tracking of unrestrained insects further revealed changes in movement patterns and within-plant preferences associated with vibrational cues alone. Overall, by integrating approaches from ecological theory and sensory ecology, this study provides mechanistic insights into how predator-associated signals may be exploited as perception-based, environmentally compatible tools to modulate spittlebug behavior and potentially limit *X. fastidiosa* transmission.

P98. *Xylella fastidiosa* subs. *multiplex* in *Philaenus spumarius* mouthparts: infection title and persistence

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The plant pathogen *Xylella fastidiosa* subsp. *multiplex* infects numerous host plants, causing symptoms collectively known as scorch diseases. In 2018, sequence type 87 was reported in Tuscany in Mediterranean shrubs and trees. Spittlebugs (Hemiptera: Aphrophoridae) are recognized as the main vectors of *X. fastidiosa* in Europe; however, their role in transmitting this subspecies remains poorly understood. This study evaluated the ability of *Philaenus spumarius* to acquire *X. fastidiosa* subsp. *multiplex* from an artificial diet under controlled experimental conditions. Insects were exposed for 6 h (Acquisition Access Period, AAP) to an artificial diet supplemented with three different concentrations of bacterial colony-forming units (CFUs). The most effective acquisition conditions were achieved by confining individual insects in 1.5 ml plastic vials with the head oriented upward, allowing feeding through a Parafilm® membrane covering a drop of artificial diet containing a known bacterial concentration. Following exposure, insects were collected over a 10-day period, and the presence of the bacterium was assessed by qPCR according to the protocol described by Harper et al. (2010; Erratum 2013). The results demonstrated that *P. spumarius* can acquire *X. fastidiosa* from an artificial diet and retain the bacterium within its mouthparts for more than 10 days, although with a progressive reduction in bacterial load. Data were plotted on a semi-logarithmic scale and linearized to estimate regression parameters. In all tested bacterial concentrations, *X. fastidiosa* did not show exponential growth within the vector, contrary to assumptions commonly used in epidemiological models. Instead, bacterial loads decreased exponentially over time ($\beta < 0$ for all concentrations), approaching an asymptotic equilibrium value dependent on the initial bacterial concentration. In all treatments, the equilibrium state was reached approximately 120h (5 days) after exposure.

P99. Elucidating the drivers of phagodeterrance for *Philaenus spumarius* induced by the delivery of a plant extract in the xylem steam

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The chemical basis of insect vector–host plant interactions is a key factor shaping the dynamics of vector-borne plant pathogen spread. The xylem-limited bacterium *Xylella fastidiosa*, transmitted in Europe by the meadow spittlebug *Philaenus spumarius*, threatens agricultural systems and cultural landscapes worldwide. Current control strategies rely mainly on chemical treatments to reduce vector abundance; however, pesticides often provide inconsistent results in limiting pathogen spread and raise ecological and toxicological concerns. An alternative, yet poorly explored, approach to reducing transmission risk is decreasing host plant palatability for insect vectors. Previous experiments showed that injection of a Eucalyptus globulus extract (EGe)—a compound with no lethal or sublethal effects on *P. spumarius* when applied topically or residually—into the xylem stream alters sap chemistry and reduces vector activity and inoculation rates. However, the mechanistic basis of the observed phagodeterrence remains unclear, particularly whether it results from the compound itself or from plant-mediated responses triggered by the injection. Here, we further investigated the mechanisms underlying this effect. By combining choice assays with the Electrical Penetration Graph (EPG) technique, we evaluated dose-dependent responses to increasing EGe concentrations (20, 40, 80, and 150 µL/mL). None of the tested doses differed significantly in their effects on host acceptance or probing behavior. Time-course choice tests indicated that xylem chemical homeostasis was restored approximately one month after injection. We also assessed whether the deterrent effect was attributable solely to the active compound or to interactions with formulation coformulants. Insects exposed to plants treated with coformulants alone exhibited a similar, albeit weaker, response compared with those on EGe-treated plants, indicating that the phagodeterrent effect cannot be attributed exclusively to the active compound. By integrating untargeted metabolomic and transcriptomic analyses, we show that the observed deterrence is likely mediated by a plant stress response triggered by EGe injection, resulting in increased concentrations of organic acids in the xylem sap. These findings provide new insights into the mechanisms underlying plant-mediated modulation of vector behavior and highlight the potential of manipulating plant chemistry as a sustainable strategy to reduce pathogen transmission.

P100. Spring soil applications of *Metarhizium brunneum* reduce *Bactrocera oleae* and *Auchenorrhyncha* populations

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Soil application of EAMa 01/58-Su strain of the entomopathogenic fungus *Metarhizium brunneum* (*EF*) targeting last instar larvae of olive fruit fly (OFF) beneath the tree canopy has been previously showed to be an effective alternative to chemicals in terms of efficacy and security for environment and human health. However, within an integrated pest management (IPM) strategy, soil application of this multifunctional EF strain could provide added values to both olive crop production and protection. This study delves into the efficacy of such soil treatments during Spring to target both emerging OFF adults and *Auchenorrhyncha* (Hemiptera) populations, known potential vectors of Xylella fastidiosa Wells. For that, three year wide-area field study was carried out within 40 hectares of olive orchards located in Alcolea (Cordoba), southern Spain. The results obtained from the study showcased promising outcomes, with the fungal strain demonstrating noteworthy effectiveness in pest control. Specifically, the application of the *M. brunneum* EAMa 01/58-Su strain resulted in a considerable 40% decrease in the OFF population when compared to untreated controls. Moreover, there was a significant reduction of over 50% in the natural populations of *Auchenorrhyncha* insects. The fungal inoculum reached basal levels approximately four months post- treatments setting at 3.74x103 conidia per g of soil. These findings underscore the potential of the EPF *M. brunneum* (EAMa 01/58-Su strain) as a viable component of IPM strategy in olive orchards, offering a sustainable alternative to conventional pesticide approaches. Moreover, the observed decline in *Auchenorrhyncha* populations suggests a broader ecological benefit by potential mitigating the spread of *X. fastidiosa*, a significant concern in the region.

P101. Dynamic core bacteriome across life stages of *Philaenus spumarius* reveals functional shifts relevant to vector ecology

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Insect microbiomes have received increased attention over the years due to their critical roles in host functions. The microbiome of *Philaenus spumarius*, the European vector for the transmission of *Xylella fastidiosa*, remains poorly studied and understood. In this work, a metabarcoding approach was employed, and the functional prediction of detected bacterial communities was performed to investigate the changes in the bacterial communities from nymphs to adults, describing for the first time the core bacteriome of this insect across different life cycle stages. The bacteriome of nymphs was revealed to be less diverse yet more abundant in functional pathways compared to adults. However, female adults show greater similarity to nymphs in terms of bacterial abundance and predicted functional pathways. A core bacteriome was identified, composed of 12 bacterial genera persistently present across all stages and samples of *P. spumarius*, with variations in abundance between life stages and sexes. Among these, *Candidatus Sulcia* and *Sodalis* were more abundant in nymphs, while Candidatus Sulcia and *Cutibacterium* were more prevalent in adults. These genera displayed distinct profiles of abundant pathways, providing essential functions to the host. Additionally, core bacteria such as *Curvibacter*, *Duganella*, *Methylobacterium* and *Pseudomonas* were found to offer similar functional profiles, enriched in functions related to host protection and response to environmental stresses. Our study highlights the importance and potential of bacteriome diversity and functions throughout the insect life stages, underscoring the dynamic nature of microbial communities in *P. spumarius*.

P102. Unlocking the cultivable microbiota of *Philaenus spumarius* and their implications for insect fitness

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The microbial community of *the Philaenus spumarius*, an insect vector of the phytopathogenic bacteria *Xylella fastidiosa*, has been studied using non-cultivable methods and focusing on the bacterial community. However, the cultivation of microorganisms is crucial for developing microbiota-derived control strategies. A simple culturomic approach was used in this work to characterize the cultivable bacterial and fungal communities associated with *P. spumarius* females and males. The effect of five isolated bacterial strains on insect progeny and morphology was then evaluated using Drosophila melanogaster as a model organism over three generations. Overall, 91 operational taxonomic units from six phyla and 38 genera were identified. Three bacterial genera (*Aeromicrobium*, *Agrobacterium* and *Williamsia*) and all nine fungal genera were described for the first time in *P. spumarius*. *Pseudomonas* and *Cladosporium* were the most abundant genera identified within bacterial and fungal communities, respectively. Most isolated strains were described as plant endophytes, thus likely acquired through feeding. The bacterial strains provided in the fly food showed variable effects on D. melanogaster progeny and morphology, depending on the type of strain, the duration of strain exposure, and the insect sex. In general, all five strains increased progeny, in particular *Curtobacterium* B26, *Rhodococcus* B27, and *Microbacterium* B79, and affected the body and wing sizes of D. melanogaster. For some strains, notably Agrobacterium B34 and *Williamsia maris* B46, these effects seem to be transgenerational. Although preliminary, these findings suggest that cultivable bacteria may play significant roles in insect fitness and highlight their potential use in managing *P. spumarius* populations.

P103. The egg parasitoid *Ooctonus vulgatus* as a Biological Control Agent of *Philaenus spumarius*: A Three-Year Field and Laboratory Evaluation in Italy

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The meadow spittlebug, *Philaenus spumarius* (L.) (Hemiptera: Aphrophoridae), is the main European vector of *Xylella fastidiosa*. Effective suppression of this vector is critical to limiting pathogen dissemination, yet current control strategies – primarily tillage and insecticide applications – can present inconsistent results. Egg parasitoids may provide a synergic biological control strategy by targeting *P. spumarius* populations prior to adult emergence and subsequent pathogen transmission, including in unmanaged areas. This study assessed the occurrence and biological traits of egg parasitoids associated with *P. spumarius* in three Italian regions (Liguria, Piedmont, and Apulia) over a three-year period through integrated field surveys and laboratory assays. Field sampling was conducted in olive groves, vineyards, and fallow areas during autumn and winter using sentinel egg deployments and direct egg collection. In total, more than 13,500 host eggs were recovered following field exposure, from which 806 specimens of *Ooctonus vulgatus* Haliday (Hymenoptera: Mymaridae) emerged. Species identification was confirmed by morphological and molecular analyses. Across all regions and sampling years, *O. vulgatus* was the only egg parasitoid detected. Parasitism was widespread but highly variable among sites and years, with mean parasitism rates ranging from 5% to 25% and parasitoid emergence reaching up to 60% in some cases. The highest field parasitism occurred on sentinel eggs oviposited directly at survey sites, indicating greater susceptibility of recently oviposited eggs. Laboratory parasitism trials supported this observation, as parasitoid emergence was recorded exclusively from freshly oviposited diapausing eggs. However, the low emergence rates from host's eggs exposed for parasitism in controlled conditions (about 3%) and the short adult lifespan (9.8 ± 4.7 days) constrain the feasibility of mass rearing. Overall, *O. vulgatus* appears to have limited potential as a standalone biological control agent against *P. spumarius*. Further research should clarify the ecological and phenological factors influencing its population dynamics and parasitism performances to evaluate its exploitation within a conservative biological control framework.

P104. First insights into *Draeculacephala robinsoni* as a potential vector of *Xylella fastidiosa* and its vibrational communication

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The North American sharpshooter *Draeculacephala robinsoni* was reported for the first time in Europe in 2022. As a xylem-feeding species, it is, by definition, potentially competent for transmitting *Xylella fastidiosa* (*Xf*). This project aims to (i) characterize the bacterium transmission efficiency between herbaceous and woody hosts, using alfalfa and grapevine as proxy source and recipient plants, and (ii) describe sharpshooter intra-specific vibrational communication to develop behavioral manipulation strategies for mitigating transmission risk. Transmission assays using both single and grouped insects revealed that *D. robinsoni* is a poor vector of *Xf*, at least on the tested host species. In trials with single insects, neither acquisition nor inoculation occurred under any of the tested combinations. In contrast, acquisition from infected grapevines was detected in group-feeding assays, although at a relatively low rate (7%). Artificial diet assays indicated differential acquisition between *Xf* genotypes, with 40% of insects testing positive for *Xf* subsp. *fastidiosa* ST1 and 19% for *Xf* subsp. *multiplex* ST43 (qPCR). However, none of the insects that acquired the bacterium from artificial diet successfully inoculated alfalfa recipient plants. Preliminary characterization of vibrational communication showed that *D. robinsoni* produces four distinct, context-specific substrate-borne signals—two emitted by males and two by females—primarily associated with mating. Female signaling was only initiated in response to male calls, and mating involved a sequence of duet establishment, partner localization, and short copulation (3–10 min). Trials aimed at using vibrational signals to disrupt insect–plant interactions and reduce transmission risk are currently ongoing. Overall, these findings provide the first insights into the potential role of *D. robinsoni* in the epidemiology of *Xf* in Europe. In addition, this study establishes the first characterization of its vibrational communication behavior, providing the basis for both improved risk assessment and the development of environmentally compatible strategies for vector management.

P105. In depth monitoring of insect vectors of *Xylella fastidiosa* in the table grape district of Apulia (Italy)

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In Apulia (Italy), *Xylella fastidiosa* subspecies *fastidiosa* (ST1), the causal agent of Pierce's disease, was recently detected on grapevine and almond in the province of Bari, in 2024, in the framework of the surveillance program carried out by the Apulia Regional Phytosanitary Service. In 2025, 12 table grape vineyards within the demarcated area were monitored from April to November to assess the density and population dynamics of juvenile and adult stages of *Philaenus spumarius* and *Neophilaenus campestris*. Juveniles were surveyed weekly on spontaneous vegetation using standardized transects. Adults were monitored using yellow sticky traps (32/hectare), arranged in two concentric rings along the tendone-training system, replaced and analysed every two weeks. This approach was complemented by sweep-net sampling of vineyard ground cover and, where present, adjacent olive or almond trees. Adults were analysed by qPCR individually or in groups of 5 specimens; those testing positive were then subjected to subspecies-specific qPCR. Spittlebug juvenile population density was extremely low, averaging 0.7 individuals m2 for *P. spumarius* and 0.6 individuals/m2 for *N. campestris*. Adults of *N. campestris* were first detected in late April, with a peak on the external traps in mid-June, and a second peak in mid-November on both external and inner traps. A similar seasonal pattern was observed for *P. spumarius*, with an earlier peak in mid-May on external traps, followed by a marked decline during summer and a resurgence in late October-early November, when captures became comparable between internal and external traps. Out of 141 insect samples analysed, *X. fastidiosa* subsp. *fastidiosa* was detected in three pools of *P. spumarius* and one pool of N. campestris. These findings indicate a consistently low population prevalence of both spittlebug species in vineyards, likely driven by intensive management practices, including frequent ground cover control and insecticide applications targeting other pests. Population peaks were observed only later in the season, following the reduction or interruption of treatments, supporting the role of vineyard management in limiting vector populations during most of the growing season.

P106. *Verrallia aucta*: parasitoid of *Philaenus spumarius*

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The spittlebug *Philaenus spumarius* is the main vector of the quarantine bacterium *Xylella fastidiosa* in olives in Apulia (southern Italy). In recent years, increasing research efforts have focused on identifying potential biological control agents targeting the juveniles or the adults, including predators, parasitoids and entomopathogenic fungi. Among these, the endoparasitoid fly Verrallia *aucta* Fallén (Diptera: Pipunculidae) is the most widely documented natural enemy of *P. spumarius*. This dipteran oviposits inside the body of newly emerged spittlebug adults, its larvae develop within the host, first inducing sterility and subsequently causing death. Specimens of *P. spumarius* were collected in several olive-growing areas across Apulia. Between 2019 and 2023, a total of 51 sites located in the provinces of Lecce, Brindisi, Taranto, Bari, and Barletta-Andria-Trani (BAT) were surveyed. To maximize the detecting of parasitoid larvae, only adults of *P. spumarius* collected between late July and November were examined. In total 2,393 specimens were dissected under a stereomicroscope to assess the presence of *V. aucta* larvae. All larvae were identified through molecular analyses using species-specific primers targeting the cytochrome c oxidase subunit I (COI) gene and the internal transcribed spacer 2 (ITS2). Second-instar larvae were also identified morphologically. All larvae were confirmed to be *V. aucta*. However, the overall parasitism rate was very low, ranging from 0% in sites located in Lecce and BAT to a maximum of 4.5% in the metropolitan area of Bari. These findings indicate that *V. aucta* is unlikely to represent an effective biological control agent of *P. spumarius* in Apulia. Nevertheless, the results underline the need for continued research aimed to identify novel, environmentally sustainable control strategies that can complement existing management measures adopted to mitigate diseases associated with *X. fastidiosa*.

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P107. Beyond polarization: Fostering dialogue and responsible innovation in *Xylella fastidiosa* research

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The management of the *Xylella fastidiosa* epidemic in olive-growing areas of Apulia has sparked more than a decade of intense debate, contestation, and polarization among scientists, policymakers, farmers, activists, and local communities. Discussions have often been framed in oppositional terms—science versus denialism, development versus 'backwardness', eradication versus coexistence—and have frequently been marked by public confrontation, mutual delegitimization, and deep-seated mistrust. This has narrowed the space for constructive dialogue and limited meaningful engagement not only with the technical, but also with the social, political, and ethical dimensions of plant disease governance. Yet, after more than ten years of conflict, it is increasingly clear that the challenge is not only to control the spread of a pathogen, but also to rebuild legitimacy and public trust while creating more inclusive forms of participation in knowledge production, innovation, and policy. Responsible innovation offers a valuable framework for this task. Originally developed to orient scientific and technological change toward socially desirable, ethically acceptable, and collectively negotiated futures, responsible innovation highlights anticipation, reflexivity, inclusion, and responsiveness as key dimensions of innovation processes. This paper applies that framework to the contentious management of *X. fastidiosa* in Apulia, asking what it would mean to govern plant disease research and innovation in ways that move beyond polarization and enable more meaningful interdisciplinary and transdisciplinary collaboration. Drawing on insights from philosophy of science and science governance studies, as well as long-term ethnographic research on the Apulian epidemic and its management, the paper examines how different actors understand risk, expertise, innovation, and responsibility, and how these differences shape conflicts over containment measures, resistant cultivars, farming practices, and competing visions for the future of olive farming in the region. We argue that responsible innovation can shift attention from the narrow question of which technical solution is most effective to the broader issue of how innovation is framed, by whom, and with what consequences for affected communities and landscapes. In doing so, the paper proposes responsible innovation as a framework for fostering dialogue and more democratically legitimate forms of plant disease governance.

P108. The *Xylella fastidiosa* epidemic in Puglia: a multidisciplinary analysis of the role of information and disinformation in managing phytosanitary emergencies

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The outbreak of the bacterium *Xylella fastidiosa* in Puglia represents one of the most complex environmental and agricultural disasters in recent European history. Beyond its biological impact, the epidemic can be considered as the “perfect case study" regarding the challenges of communication and the systemic influence of information and misinformation on public policy and crisis management. This research, within the Task 8.1 of BEXYL project, investigates the role of traditional media, public institutions, and social platforms in driving public perception, thereby affecting the epidemic's advancement and the operational effectiveness of eradication/containment efforts. The study’s core objective is to identify the critical bottlenecks in the communication chain over the past decade to define best practices and protocols for future phytosanitary or environmental emergencies. Adopting a purely qualitative methodology, the investigation utilizes expert interviews with "privileged witnesses" to ensure a deep, historical analysis of the crisis. The target sample follows a triangulation strategy, involving four key macro-categories: professional journalists (local, national, and specialized), regional phytosanitary officials, scientific researchers, and agricultural representatives/agronomists. The analytical framework centers on the "Information Value Chain" tracing how technical scientific data is mediated and received. Central to the inquiry is the conflict between institutional drivers, focused on governance and regulatory compliance, and media drivers, often dictated by newsworthiness, economic pressure (click-baiting), and emotional proximity to the landscape. The study specifically probes the "false balance" trap, where the media gave equal weight to scientific evidence and unverified alternative theories in the name of pluralism, thereby fueling public mistrust and obstructing eradication efforts. Furthermore, the research explores how institutional vacuums/gaps in communication were filled by anti-scientific narratives on social media, complicating the work of officials on the ground. By analyzing these "information short circuits" the study concludes by advocating for the permanent integration of "Crisis Communicators" within public institutions. Ultimately, the research argues that transparent, timely, and technically accessible information is not merely administrative support but an essential component of the phytosanitary prophylaxis itself.

P109. Bridging the knowledge-action gap: public perception of invasive alien species and the proximity effect of the *Xylella fastidiosa* epidemic in Apulia

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The growing frequency of biological invasions driven by globalization and climate change represents a systemic threat to biodiversity and agricultural stability. Within the framework of the PNRR Agritech project (Task 3.3.3), "Innovative strategies and networking tools for the involvement of stakeholders on plant protection", the CNR-IPSP conducted an ongoing study to evaluate public awareness and risk perception regarding Invasive Alien Species (IAS). The questionnaire "Biological Invasions, Alien Organisms, and Public Opinion", due to the initial dissemination channels, was proposed to a highly educated public. Preliminary results indicate a high level of theoretical literacy, with a vast majority of respondents correctly identifying the definition of IAS and having been previously informed through scientific or digital media. However, a significant "knowledge-action gap" was identified: while most respondents recognize the biosecurity risks of transporting biological materials, a relevant portion still admits to carrying plants or seeds during travel. This risky behaviour is compounded by a perceived lack of enforcement, as the vast majority reported never being subjected to phytosanitary controls. The study highlights a distinct "geography of perception" directly correlated with the *Xylella fastidiosa* outbreak in Southern Italy. In Apulia, *X. fastidiosa* is the most frequently cited IAS, with respondents reporting direct experiences of landscape devastation and economic loss in olive production. This "proximity effect" serves as a more powerful driver of risk awareness than generic educational campaigns. Furthermore, the survey reveals widespread scepticism toward current management strategies, with a significant portion of the sample considering institutional interventions ineffective. In conclusion, the data suggest that while the public possesses theoretical knowledge about IAS, this does not automatically translate into safe travel practices. To effectively modify and educate traveller behaviours, communication strategies must move beyond generic awareness campaigns. Interventions should focus on targeted, context-specific education regarding the severe risks of transporting biological "souvenirs", coupled with an increased visibility of phytosanitary border controls. Only by addressing this behavioural gap, the authorities can foster a sense of active responsibility and significantly reduce the human-mediated spread of invasive threats.

P110. Ecological drivers of vector-borne infections in agroecosystems: shortfalls and opportunities for the management *of Xylella fastidiosa*

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Research on the epidemic of *Xylella fastidiosa* infections in southern Europe has largely focused on pathogen detection, vector control, and host resistance, whereas ecological drivers have only received limited attention. Even assessments by European bodies such as the European Food Safety Authority have historically prioritized containment and vector suppression strategies, while a growing body of ecological and epidemiological literature is strengthening evidence of the role of ecosystem simplification and perturbation as drivers of vector-borne plant pathogens. Emergency measures currently adopted in Apulia region, particularly broad-scale weed removal and insecticide treatments, have been raising serious concerns regarding their implications for ecosystem stability and functionality. These management strategies can inadvertently alter trophic interactions and vector behaviour in ways that favour pathogen transmission, therefore risking being ineffective or even counterproductive. An early removal of herbaceous layer can trigger vector shifts from preferred hosts to tree crops, promote premature adult dispersal, and increase the probability of xylem probing on trees. Similarly, broad-spectrum insecticides can produce multiple unintended outcomes that increase infection pressure, by disrupting predator and parasitoid communities, and enhancing recolonization of simplified environments after treatments. Besides highlighting the shortfalls associated with current management strategies, field observations based on resource complementation and habitat-mediated dynamics can provide unexplored pathways to reduce transmission pressure while enhancing agroecosystem resilience. Alongside pathogen-based approaches, future research and policy should prioritize the adoption of nature-based solutions at the ecosystem scale, such as managed ground cover, behavioural barriers, sustainable biological control and landscape diversification, to achieve effective and lasting control of vector-borne plant diseases.

P111. Development of a decision-support system for efficient and sustainable management of *Xylella fastidiosa*

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Xylella fastidiosa (*Xf*) is a xylem-limited plant pathogenic bacterium native to the Americas and first officially detected in Europe in 2013 in Apulia (southern Italy). Since then, the pathogen has been recorded across several European regions, causing severe and economically significant diseases such as Pierce's disease in grapevine, olive quick decline syndrome, and almond leaf scorch disease. As epidemics become established and widely distributed in some European regions, eradication of the pathogen becomes unfeasible, and management priorities must shift from emergency eradication and containment measures toward long-term, adaptive Integrated Pest Management (IPM) strategies. Within the Horizon Europe BeXyl project, a user-oriented Decision Support System (DSS) is being developed to support farmers, nurseries, and plant health authorities in implementing effective and sustainable IPM strategies against *Xf* in European agricultural systems. The DSS focuses on the three main affected crops in Europe (i.e., almond, grapevine, and olive) and organises management strategies following the IPM principles of prevention, monitoring, and intervention, combining multiple control methods. The DSS is being developed through a structured methodology that includes: (i) identification and classification of IPM options currently available in Europe targeting the pathogen, vector, host plants, and landscape components; (ii) definition of key contextual factors influencing management decisions in different agricultural and epidemiological scenarios; (iii) development of candidate IPM schemes through the strategic combination of management options under varying levels of management intensity; and (iv) technical evaluation through structured expert judgment to compare alternative strategies and identify the most suitable schemes. The DSS is organised through a decision-tree framework that integrates contextual information provided by users to generate tailored recommendations on appropriate IPM options and schemes, together with implementation guidance adapted to the user's operational conditions. The DSS is being designed to be intuitive, accessible, and user-friendly for the end users. By combining scientific evidence, expert knowledge, and context-specific information, the DSS aims to support informed decision-making for the effective management of *Xf* epidemics and to promote more resilient and sustainable agricultural production in affected regions of Europe.

P112. Mapping Research Gaps on *Xylella fastidiosa*: Toward a Socio-Ecological Perspective for Apulia

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(See thematic session 5.6a)

P113. Beyond the pathogen: why the ecology and evolution of *Xylella fastidiosa* matter

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The ecological role of *Xylella fastidiosa* in the ecosystems where it likely evolved remains poorly understood. Despite intense scientific attention following its emergence in Europe, research has focused predominantly on agricultural systems and on the economic impacts of the diseases it causes. This strong bias toward agroecosystems has shaped our perception of *X. fastidiosa* primarily as an agricultural pathogen, leaving its ecological role in natural plant communities largely unexplored, particularly in the hyperdiverse Neotropical forests where many of its lineages are thought to have originated. As a consequence, we still have very limited knowledge of the ecological function, prevalence, and diversity of *Xylella* in its native ecosystems. Studying the bacterium in these environments presents substantial practical challenges: many potential hosts are canopy-forming trees in dense tropical forests, where sampling requires specialized access to upper forest strata, and infections in wild plants may often be asymptomatic or weakly expressed, making detection difficult without molecular tools. These ecosystems are also characterized by extraordinary diversity of potential plant hosts and xylem-feeding insect vectors, potentially generating complex transmission networks that may sustain a much greater diversity of *Xylella* lineages than currently recognized. In this presentation, I argue that understanding the emergence and spread of *Xylella* requires moving beyond an exclusively agricultural perspective and considering the ecological and evolutionary context in which the bacterium evolved. Recent studies suggest that the genus *Xylella* may be far more diverse—and possibly much older—than previously assumed. In this context, evolutionary processes such as variation along the symbiotic continuum from mutualism to pathogenicity, host range evolution, and phylogenetic constraints may strongly influence the emergence potential of different *Xylella* lineages. Recognizing these processes may be critical for anticipating future invasion risks in Europe. By proposing a set of speculative ecological and evolutionary hypotheses, I aim to stimulate new research directions that integrate pathogen biology with the complexity of natural ecosystems.

P114. A hyperspectral phenotyping platform for early detection of *Xylella fastidiosa* infection in woody hosts

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(See thematic session 7.7a)

P115. Horizon Scanning as a as an integrated approach on monitoring *Xylella fastidiosa*

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(See thematic session 6.8b)

P116. Volatile and metabolomic profiling of *Xylella fastidiosa* subspecies reveals strain-specific signatures with potential diagnostic value

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Xylella fastidiosa is a xylem-limited bacterial pathogen responsible for severe diseases affecting several economically important crops. Identifying biochemical signatures associated with different subspecies may support the development of rapid diagnostic and monitoring tools. In this study, the volatilome and metabolome of three genetically distinct isolates belonging to subsp. *pauca* (ST53), *fastidiosa* (ST1), and *multiplex* (ST87) were investigated using complementary analytical approaches. Volatile organic compounds (VOCs) emitted by viable cultures of the three *X. fastidiosa* strains and by the heterologous bacterium Pseudomonas marginalis were analysed using dynamic headspace sampling coupled with thermal desorption and gas chromatography–mass spectrometry (DHS-TDU/GC-MS). Distinct volatilomic fingerprints were observed among the analysed strains. Differences were mainly associated with variations in the relative abundance of specific metabolite classes, including alcohols, ketones, hydrocarbons and sulfur-containing compounds. Multivariate statistical analyses and machine-learning classification models based on VOC profiles revealed clear clustering of the *X. fastidiosa* strains, whereas *P. marginalis* showed a markedly different volatile profile, confirming the strong discriminatory potential of volatilomic data. Complementary metabolomic analyses of methanolic extracts from the same bacterial viable cultures performed by HPLC-DAD-QToF-MS further highlighted metabolic differentiation among the analysed strains at the intracellular level, while extracellular profiles were largely dominated by amino acid-related metabolites. These results indicate that integrated volatilomic and metabolomic profiling can capture subspecies-level metabolic variability in *X. fastidiosa*, providing candidate biochemical signatures that may support the development of innovative diagnostic and monitoring strategies for early detection of the pathogen. Ongoing work will extend these analyses to infected and non-infected olive leaves from resistant and susceptible genotypes to identify plant-associated volatile and non-volatile biomarkers linked to infection status.

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P117. Mitigating *Xylella fastidiosa* Risks in Türkiye Through Phytosanitary Strategies

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Xylella fastidiosa is a bacterial pathogen that poses a significant threat to natural habitats and many plant hosts cultivated largely in Türkiye. Although the pathogen is currently categorized as absent in the country, Türkiye remains at high risk due to the extensive cultivation of primary hosts—including *Olea europaea* (olive), *Vitis vinifera* (grape), *Citrus spp*., *Prunus dulcis* (almond), and various stone fruits and ornamentals. This vulnerability is compounded by the presence of indigenous xylem-feeding insect vectors, a high volume of international plant material imports, and favorable climatic conditions for pathogen establishment, which could lead to catastrophic agricultural yield losses and habitat degradation. To mitigate these risks, comprehensive legislative measures have been implemented. In alignment with Regulation (EU) 2020/1201, the "Regulation on the Control of *Xylella* Leaf Scorch" was promulgated in the Official Gazette on December 14, 2024. Following the initial outbreaks in Italy, the General Directorate of Food and Control integrated *X. fastidiosa* into the mandatory national survey list. The National *Xylella* Leaf Scorch Project has been carried out, involving seven specialized governmental research institutes to facilitate early detection and vector identification across all geographical regions of Türkiye. Since 2020, surveillance activities have utilized risk-based approach with four distinct risk categories (RC) (RC I: Agricultural areas where main crops such as olive, almond, grape, citrus, stone fruits were grown (olive, almond, grapevine, citrus, and stone fruits), RC II: Natural and semi-natural habitats, specifically maquis and heathland, RC III: Commercial nurseries and ornamental plant production places/markets RC IV: Urban landscapes and recreational areas.) defined . Between 2022 and 2025, over 5,000 plant samples were collected alongside various potential insect vectors which were used for species-level identification and pathogen testing. Both plant and insect samples were screened using internationally validated real-time PCR protocols. All analyses yielded negative results, confirming the absence of the pathogen in the country. In response to an official inquiry by the European Commission's Directorate-General for Health and Food Safety (DG SANTE) regarding Articles 28 and 29 of Regulation (EU) 2020/1201, Türkiye formally notified the Commission of its pest-free status by submitting comprehensive technical report on risk-based surveys supported by laboratory analyses in December 2022. Consequently, the European and Mediterranean Plant Protection Organization (EPPO) declared Türkiye as a Pest-Free Area (PFA). This status is vital for maintaining international trade, as it effectively removes phytosanitary barriers for plant exports to EU member states Continuous public awareness activities/campaigns regarding the pathogen and its vectors remain active to ensure robust community engagement in maintaining national plant health.

P118. Sentinel plants as tools for tracking insect vectors of *Xylella fastidiosa* in olive orchards

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In pathosystems involving vector-borne pathogen transmission, monitoring the occurrence of vectors is essential for predicting the introduction and spread of pathogens within production systems, particularly through the assessment of insect infectivity. In the case of the *Xylella fastidiosa* (*Xf*) pathosystem, it is well known that certain plant species are more attractive to vectors and may also serve as reservoirs for the bacterium. In this study, we monitored the presence and diversity of xylem sap-feeding sharpshooters (Cicadellinae and Cercopoidea) using sentinel plants in an experimental olive grove located in the Serra da Mantiqueira mountains in Southeastern Brazil (Minas Gerais, State). Plant species including *Citrus sinensis*, *Duranta erecta*, *Polygala sp*., *Olea europaea*, and *Nerium oleander* were strategically arranged in blocks across different locations within an olive orchard with a known history of *X. fastidiosa* occurrence. The objective was to determine which plant species most frequently and earliest supported the natural occurrence of both the insect vectors and the pathogen. Visual assessments and collections revealed a higher number of insects on Polygala sp. (20 out of 47 individuals). Minor numbers were recorded on *N. oleander* (n = 13), *C. sinensis* (n = 7), *D. erecta* (n = 4), and *O. europaea* (n = 3). Among the collected specimens, the following sharpshooter species were identified: *Bucephalogonia xanthophis* (n = 2), *Scopogonalia sp*. (n = 12), and *Macugonalia leucomelas* (n = 19). The remaining specimens have not yet been identified. Most specimens (39 out of 47) were found in the block boarded by native forest, highlighting the potential role of alternative host plants as reservoirs for sharpshooters. Analyses to detect the presence of *X. fastidiosa* in sentinel plants and to assess insect infectivity are currently underway. Based on the results obtained so far, Polygala sp. proved effective in attracting sharpshooters and may serve as a useful target plant for monitoring *X. fastidiosa* vectors, particularly in regions where the pathogen is not yet established or in newly developed orchards within endemic areas. Additionally, greater attention should be given to host plants located near native forests during orchard inspections.

P119. Temperature-driven dynamics of colonization and multiplication in *Xylella fastidiosa* subsp. *pauca*

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Global warming and extreme weather events have raised growing concerns regarding agricultural production and pest's incidence. In the case of vector-borne diseases, such as caused by *Xylella fastidiosa* (*Xf*), these changes have even more significant and unpredictable effects. Understanding how environmental factors, especially temperature, influence the pathogen's ability to colonize is essential for predicting epidemic outbreaks in the context of climate change. In this study, we are evaluating, under controlled conditions, the influence of different temperature regimes on: #1. Acquisition and transmission of *Xf* subsp. *pauca* (*Xfp*) by the vector *Macugonalia leucomelas* feeding on infected olive trees (Olea europaea) and transmitting it to Catharanthus roseus, and #2. Transmission and colonization of the bacterium inoculated by needle into C. roseus. The inoculations were performed using a bacterial suspension of *Xfp* ST16 isolated from an olive tree in Southeast Brazil) and adjusted to high inoculum concentration (OD600 of 1). The suspension was inoculated into the plants using the pin-pricking method, and plants were then placed in growth chambers set at 14/22°C, 18/28°C, and 22/32°C (16 h day/8 h night). Both infection and colonization were monitored by qPCR (Harper et al., 2010) at 45, 70, and 90 days after inoculation (DAI). At 45 days post-inoculation, no significant effect of temperature on infection efficiency was observed, with an average success rate of 88.9 ± 0.11% regardless of the treatment. However, a significant effect of temperature on bacterial titer was observed after 70 DAI. In plants maintained at 22/32°C, there was a significant decline in pathogen concentration at 70 ($p = 0.011$) and 90 ($p = 0.015$) DAI compared to plants maintained at 14/22°C. In contrast, for plants maintained at the median temperature (18/28°C), the reduction in bacterial titer was not significant. These results suggest that exposure to high temperatures may limit the pathogen's multiplication within the host, but not the infection. Based on these results, it can be inferred that scenarios involving prolonged periods of temperatures above 32°C may restrict the bacterial titer in the plant, with potential implications for predicting disease progression. Data regarding population dynamics in olive trees, as well as vector acquisition and transmission, are currently under analysis and may further support predictive models of *Xf* dispersion in the face of climate change.

P120. Modelling *Xylella fastidiosa* infection dynamics in grapevines: a physiologically based approach

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Xylella fastidiosa subsp. *fastidiosa* (*Xff*), the causal agent of Pierce's Disease (PD) in grapevine, is a major threat to viticulture worldwide. However, the processes governing infection establishment, persistence, and recovery within grapevine hosts remain incompletely understood. In this study, we developed a novel physiologically based infection model that explicitly describes the population dynamics and within-plant spread of *Xff* in grapevine plants as a function of temperature and host phenology. The model represents grapevines as structured hosts composed of two interacting tissue compartments: permanent woody tissues and seasonally renewed annual tissues. Bacterial growth, mortality, and translocation between compartments are modelled mechanistically through temperature-dependent functions and seasonal variation in tissue availability, enabling the explicit simulation of growing-season dynamics, winter dormancy, pruning, and cold-induced recovery. The model is formulated as a stochastic, physiologically based framework using coupled Kolmogorov forward equations, allowing representation of heterogeneity in bacterial loads among infected plants and the probabilistic outcome of chronic infection or recovery. Winter recovery is driven by high-resolution cold degree day (CDD) accumulation function controlling bacterial mortality in permanent tissues, whereas upward bacterial movement governs seasonal colonization of annual tissues and detection dynamics. Model parameters were estimated using experimental and field observations. Simulations were conducted across 21 climatically distinct locations to evaluate infection outcomes under different temperature regimes, inoculation timing, and inoculation pressure. Model simulations successfully reproduced key epidemiological patterns reported in empirical studies, including seasonal detection dynamics, temperature-dependent establishment of chronic infection, winter recovery, and multi-year progression of bacterial populations. The framework highlights the critical role of interactions between permanent and annual tissues in shaping PD dynamics and provides mechanistic insight into how climate modulates infection persistence. By explicitly linking within-host bacterial processes to local climatic conditions, the model provides a novel tool to explore PD risk, evaluate future climate scenarios, and support the optimization of surveillance and management strategies at fine temporal and spatial scales.

P121. National Surveillance Framework for *Xylella fastidiosa* in Kosovo

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Xylella fastidiosa has been identified as a key phytosanitary threat in Europe where it is responsible for significant economic impacts on various crops particularly in the Mediterranean region. In Kosovo, *X. fastidiosa* is classified as a priority pest and is included in the National Monitoring and Surveillance Program of the Phytosanitary Inspectorate of the Food and Veterinary Agency. As olive crops are not cultivated in Kosovo, all *Xylella fastidiosa* surveillance and monitoring activities are focused mainly on grapevine (*Vitis vinifera* L.), which represents the most important agricultural sector in the country. Annual systematic field inspections are undertaken to identify symptoms indicative of *Xylella fastidiosa* infection, such as leaf scorch and dieback. As part of the national monitoring plan, the Phytosanitary Inspectorate collects approximately 50 plant samples per year from different viticultural regions. These samples are delivered for laboratory analysis to the Plant Protection Laboratory at the Kosovo Institute of Agriculture. To ensure early detection and rapid response in case of pathogen presence, diagnostic procedures of collected samples is accomplished through the use of standardized diagnostic protocols developed by the European and Mediterranean Plant Protection Organisation (EPPO). To date, there are no confirmed *Xylella fastidiosa* cases within the territory of Kosovo. However, continuous monitoring, capacity building, and complying with Phytosanitary Regulations of the European Union will be the key components of the ongoing National Plan to stop this quarantine pest from introducing itself into Kosovo and spreading within the country.

Keywords: *Xylella fastidiosa*, surveillance, phytosanitary monitoring, grapevine, Kosovo

P122. A two-year follow-up of four coffee plantations with *Xylella fastidiosa* in endemic conditions in Costa Rica

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Coffee plantations in Costa Rica are distributed across eight regions; most coffee is produced in the highlands at elevations of approximately 1000 meters above sea level in the central intermountain valley. These regions are embedded within a wide variety of climate zones and ecosystems and consequently harbor great biodiversity. These abiotic and ecological pressures are evidenced by the presence of insect and bacterial diversity within coffee-producing landscapes. The *Xylella fastidiosa*–coffee pathosystem is composed of a susceptible host, introduced to Costa Rica in the late 1800s, and the settlement of coffee plantations favored exposure to and interaction with endemic *X. fastidiosa* subsp. *fastidiosa* as well as native circulating vectors. Different coffee varieties and plant species as shade, with undetermined susceptibility to *X. fastidiosa* have been utilized throughout the years, although the impact it may have on the pathosystem remains unknown. In Costa Rica, *X. fastidiosa* is widely distributed, and exhibits broad diversity among circulating strains, however, only a small number of plants develop mild to severe symptoms of "crespera", with no reported impact on coffee production, despite plants being heavily colonized. In this work, we followed four coffee plantations located in Carrizal and Tambor (north Central Valley), San Francisco (highland south Central Valley), and Quebradilla (east Central Valley). Each coffee plantation has different management practices and the presence of different abiotic and biotic traits. Thirty coffee plants were selected within each coffee plot and surveyed every six months, encompassing two dry and two rainy seasons. We evaluated the presence of crespera disease symptoms, *X. fastidiosa* detection, leafhopper population, the presence of other coffee diseases, temperature, humidity, ground covers, site description, and management practices. All data were integrated and compared. Overall, this study contributes to the understanding of *X. fastidiosa* -coffee pathosystem under endemic conditions in Costa Rica and provides insights into specific traits that are relevant for surveillance.

P123. *Xylella fastidiosa* in Portugal: Current Epidemiological Status and Spatiotemporal Dynamics (2019–2026)

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Since the first detection of *Xylella fastidiosa* in Vila Nova de Gaia (Portugal) in 2019, the country has been managing a progressively complex phytosanitary situation. Based on official surveillance data from the National Plant Protection Organization, the Directorate-General for Food and Veterinary (DGAV), in collaboration with the Institute for Nature Conservation and Forests (ICNF), this work evaluates the multi-year (2019-2025) implementation of eradication measures in several areas, alongside containment strategies, with particular attention to high-density areas such as the Porto Metropolitan Area. The epidemiological scenario has been evolving with the expansion of Demarcated Areas (DAs) over the past four years. At the beginning of 2026, there was a further expansion of DAs, one in the Alentejo region and six in the Central region. In contrast, historical outbreaks in Tavira (Algarve) and Palmela (LTV) were officially declared eradicated. This shifting dispersion highlights shared regional risk profiles and agro-ecological continuities, establishing a critical framework for long-term territorial evaluation. *Xylella fastidiosa* subsp. *multiplex* ST7 was the first lineage detected, associated with ornamental and native hosts (*Lavandula* spp., *Cytisus* spp., *Quercus* spp.) and characterized by a general absence of significant symptomatic decline. More recently, the detection of *X. fastidiosa* subsp. *fastidiosa* has revealed its widespread geographical occurrence across the Iberian Peninsula, far surpassing the known distribution of subsp. multiplex. Surveillance data show an overall positivity rate of only ~2.33% across the study period, with detections predominantly concentrated in urban (6.41%), forest, and semi-natural environments. Crucially, the cumulative infected area stands at 361 hectares, whereas the total demarcated area encompasses 186,056 hectares, of which 18.69% overlaps with legally protected areas. This substantial overlap highlights the ecological sensitivity of the regulated territory. Furthermore, the widespread occurrence of the pathogen across the Iberian Peninsula underscores the critical importance of continuous monitoring and cross-border information exchange. These findings indicate a long-term presence of *X. fastidiosa* in Portugal, likely originating from multiple historical introduction events. Furthermore, the limited symptomatic expression currently observed in major economic crops suggests that contemporary infections are predominantly associated with non-agricultural, tolerant reservoirs. Consequently, this analysis underscores the importance of carefully balancing strict eradication protocols with regional socio-economic and ecological sustainability. We conclude that future phytosanitary frameworks would benefit from being progressively tailored to this epidemiological reality, exploring harmonized and flexible containment approaches to optimize risk mitigation in key sectors—such as viticulture, olive, almond, citrus, and cork production—while supporting environmental and conservation objectives.

Keywords: Plant Health, Epidemiology, subsp. *fastidiosa*, subsp. *multiplex*, outbreaks.

P124. Artificial inoculations reveal new potential hosts of *Xylella fastidiosa* subsp. *pauca*

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Switzerland is currently free of *Xylella fastidiosa*. However, due to the potential damage it could cause to crops, ornamental and forest tree species, it is classed as a priority quarantine organism. To be better prepared for a possible introduction of *X. fastidiosa*, and to improve its surveillance, woody plant species relevant for Swiss agriculture and forestry were artificially inoculated with different *X. fastidiosa* subspecies in a biosafety facility. Seven plant species were selected (*Vitis vinifera*, *Prunus avium*, *Prunus armeniaca*, *Olea europaea*, *Rubus fruticosus*, *Quercus petraea* and *Fagus sylvatica*), and inoculated with *X. fastidiosa* subsp. *fastidiosa*, subsp. *multiplex* and subsp. *pauca*. Leaves were sampled 4 and 11 months after the artificial inoculation and analysed by qPCR. Symptoms were visually assessed at the same timepoints by three collaborators independently. At the first sampling, *X. fastidiosa* could be detected in 6-33% of leaves of *V. vinifera*, *O. europaea*, *R. fruticosus*, *Q. petraea* and *F. sylvatica*, but not in any sample of the two *Prunus* species. Surprisingly, subsp. *pauca* was able to infect 3 out of 5 *F. sylvatica* seedlings, 3 out of 5 *Q. petraea* and 1 out of 5 *R. fruticosus*, in addition to all the *O. europaea* plants. However, 11 months after the inoculation and after one winter, the infection in most of the above-mentioned plants was lost, with only one *O. europaea* and one *Q. petraea* still testing positive. Symptoms' assessment was difficult at the first sampling timepoint, as *X. fastidiosa* symptoms were very similar to the natural senescence of the plant in autumn. In the few plants that still tested positive for *X. fastidiosa* 11 months after the inoculation, symptoms were mostly visible and typical for *X. fastidiosa*. Our results indicate that the host range of *X. fastidiosa* subsp. *pauca* could be broader than previously known and could comprise also deciduous woody species such as *F. sylvatica*. Moreover, visible symptoms could be an unreliable indicator of an infection, especially for deciduous plants towards the end of the growing season.

P125. From static list to predictive open-data tool: the applicative evolution of the EFSA *Xylella spp*. host plant database

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Since the first European outbreak of *Xylella fastidiosa* in 2013, the EFSA *Xylella spp*. host plant database has evolved from a simple inventory into a dynamic, open-access resource essential for risk assessment and management. The latest update (Version 13), covering literature up to June 2025, highlights this significant evolution. The database now encompasses 728 host plant species (464 confirmed by highly reliable methods, Category A), 319 genera, and 91 families, integrating comprehensive data on *X. fastidiosa* subspecies, sequence types, geographic distribution, and plant tolerance or resistance traits. Beyond serving as a scientific repository, the database is actively utilized by international research initiatives. For instance, the Horizon Europe BeXyl project employs the database to categorize host plants based on their habitus, propagation methods, and economic importance for the nursery sector. Feeding such agronomic and commercial data back into the EFSA database could substantially enhance its operational value, bridging the gap between scientific reports and field realities. Most importantly, this evolution paves the way for the database to become a proactive predictive tool. By intersecting its extensive host range data with regional flora inventories and nursery trade statistics, risk managers can implement targeted, preventive monitoring of high-risk species in disease-free territories. This integration enables the assessment of potential ecological impacts on specific geographic areas based on existing vegetation, as well as the evaluation of economic consequences for various nursery districts. Hopefully, transitioning from a descriptive catalogue to an applicative predictive open-data tool could empower phytosanitary authorities and stakeholders to anticipate *Xylella* outbreaks and tailor phytosanitary measures, safeguarding European agriculture and biodiversity.

P126. Survey of *Xylella fastidiosa* and *Verticillium dahliae* in olive orchards in Argentina for epidemiological studies

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In Argentina, olive production is affected by major phytosanitary problems, particularly in La Rioja province, where *Verticillium dahliae* non-defoliating pathotype has been present for more than four decades. *Xylella fastidiosa* subsp. *pauca* was detected in late 2013 in the Arauco and Castro Barros departments associated to a disease similar to Olive Quick Decline Syndrome (OQDS) in Italy. Both pathogens colonise the xylem, impair water transport, and may induce decline, wilting, branch dieback and, in severe cases, tree death. These impacts are especially relevant for cv. Arauco, the only Argentine olive cultivar included in the World Catalogue of Olive Varieties, which is widely valued for its dual-purpose but is highly susceptible to both pathogens. In 2025, surveys were conducted in four olive orchards in Aimogasta and in one INTA cultivar collection. More than 2,000 olive trees of cvs. Arauco, Arbequina, Hojiblanca and Manzanilla fina were assessed for symptoms associated with these pathogens using a canopy-based severity scale (SS) from 0 to 4. A total of 194 samples (2%) were analysed by the Harper qPCR assay for *X. fastidiosa* and the Bilodeau qPCR assay for V. dahliae. Subspecies assignment for *X. fastidiosa* was performed using the Dupas qPCR assay. Visual observation yielded the following results: 20% of the plants showed no symptoms (SS=0), and 80% showed symptoms consistent with ODDS (32.3% with SS=1, 6.7% with SS=1.5, 24.6% with SS=2, and 16.4% with SS=3). Of the 194 symptomatic trees analyzed 80,92% were positive for X. fastidiosa in Harper qPCR, 65.98% for *X. fastidiosa* in Dupas qPCR, whereas 1% were positive for both pathogens. Among the *X. fastidiosa*-positive samples, 74,4% were assigned to subsp. *pauca*, 25.6% could not be assigned to any subspecies because bacterial titres were below the detection limit of the Dupas, assay which deserves further research. These results confirm the widespread presence of *X. fastidiosa* in surveyed olive orchards in La Rioja and provide a basis for further epidemiological studies under local production conditions. Financed by Projects 'BeXyl–Beyond *Xylella*, Integrated Management Strategies for Mitigating *Xylella fastidiosa* impact in Europe (grant 101060593, HORIZON-CL6-2021-FARM2FORK-01)' and 'Disciplinary Project 2023-PD-L03-I084. Biotic and abiotic stresses in plants. Physiological and pathological studies for the design of improvement and management strategies''

P127. Update of 10 years of *Xylella fastidiosa* official survey in France

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In France, only the multiplex subspecies of *Xylella fastidiosa* (*Xf*) has been detected in the natural environment in the Corsica, Provence-Alpes-Côte d'Azur (PACA) and Occitania regions. *Pauca* subspecies was reported, then eradicated in one outbreak in Menton (PACA) (Cunty et al., 2026). Sequence-Types ST6 and ST7 have been identified in the 3 regions, in contrast to the more localized ST88 (Var) and ST89 (Alpes-Maritimes) (Cunty et al., 2022). Although the host range of *Xf* is very broad, it differs between regions. Outbreaks in the PACA region are now well characterized and remain similar to those identified 10 years ago. By contrast, new infected areas are reported each year in Occitania. In 2025, *Xf* was detected for the first time in Occitania on the sap-sucking insect *Lepyronia coleoptrata*. The subspecies multiplex ST6 was identified, consistent with the infected plants samples of the same area. *L. coleoptrata* is widespread in Europe but its population density is very low compared to *Philaenus sp*. or *Neophilaenus sp*. To date, no studies have been conducted on its potential role in *Xf* transmission. Official detection of *Xf* in plant and insect samples is based on the real-time PCR developed by Harper et al. (2010) and the identification of the subspecies on the tetraplex PCR (set n°5) developed by Dupas et al. (2019). Since 2026, the “Dupas” tetraplex has been used as the initial test to confirm positive samples in the detection scheme by the French National Reference Laboratory, enabling both detection and subspecies identification in a single procedure. In cases where the tetraplex “Dupas” fails, simplex PCR (Dupas and/or Harper) and/or Multilocus Sequence Typing (MLST, Yuan et al., 2010) are used to obtain a final result. The movement of specified plants from non-infected area is determined by the specific requirements for plants posing the highest risk as defined in regulation EU2020/12/01 (Article 25.2 revised). Consequently, lots of Coffea sp., Lavandula angustifolia, *L. dentata*, *L. x intermedia*, *L. latifolia*, *L. stoechas*, Nerium oleander, *Olea europaea*, *Polygala myrtifolia*, *Prunus dulcis* and *Salvia rosmarinus* may only be moved if tested for the presence of *Xf*. The number of samples that can be pooled is a key parameter affecting detection sensitivity mainly on plant matrices rich in inhibitors as *Lavandula sp*. and *Salvia sp*. This issue requires further investigation.

P128. Rapid volatilomic screening for *Xylella fastidiosa*: towards portable tools for phytosanitary surveillance

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(See thematic session 6.8a)

P129. Surveillance and Risk Assessment of *Xylella fastidiosa* in Montenegro in 2025

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The phytopathogenic bacterium *Xylella fastidiosa* poses a high risk of introduction, establishment, and spread in Montenegro, potentially causing substantial agricultural losses and damaging public landscapes. Key risk factors include the intensive import of host plants, international trade, and heavy tourist traffic, which may facilitate unintentional pathogen introduction. Suitable climatic conditions, the widespread presence of host plants, and the occurrence of the efficient vector *Philaenus spumarius* may further support its establishment and spread. In 2025, phytosanitary inspectors conducted visual inspections and sampling of host plants across Montenegro as part of a targeted surveillance program, focusing on high-risk locations in the central and southern parts of the country. These included importers and distributors of planting material, public areas (e.g., newly established parks, greened boulevards, resorts, and roadsides), newly planted perennial host plant plantations at registered primary producers, unregistered hobbyist producers, and registered producers of planting material. A total of 81 locations were inspected nationwide, with the majority located in public areas. In total, 96 samples were collected, with approximately equal numbers taken from symptomatic and asymptomatic plants. Most samples originated from public areas, and the most frequently sampled host plant was the olive. All samples tested negative for *X. fastidiosa* using real-time LAMP analysis. To further strengthen surveillance *Xylella fastidiosa*, Montenegro should enhance its plant health system by training phytosanitary inspectors in sampling procedures and identification of risk locations; educating producers, citizens, and tourists about the risks associated with *Xylella fastidiosa*; adopting additional molecular methods to enable subspecies-level identification; and implementing statistically designed surveys to increase the number of analysed samples. Although *Xylella fastidiosa* has not been detected so far in Montenegro, the implementation of continuous surveillance and mitigation measures remains critical to address this persistent phytosanitary threat.

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P130. Integrating multi-method diagnostics across botanic gardens to detect emerging diversity of *Xylella fastidiosa*

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Effective surveillance of *Xylella fastidiosa* requires integrated analytical approaches capable of detecting infections across diverse hosts and resolving pathogen diversity at high resolution. Within the BeXyl project, sentinel plots and botanic gardens are jointly used as complementary surveillance systems, enabling both controlled epidemiological monitoring and broad host-range screening. Sentinel plots may provide longitudinal evidence of pathogen establishment, host susceptibility, and spatiotemporal dynamics under field conditions, including detection in both plant hosts and insect vectors and confirmation of systemic colonisation and pathogen movement. In parallel, botanic gardens enable targeted sampling across taxonomically diverse and often asymptomatic hosts, expanding the detectable host range beyond agricultural systems. Within BeXyl project samples from botanical gardens were analysed using qPCR, digital PCR, and multilocus sequence typing (MLST). This approach enabled sensitive detection, robust confirmation, and high-resolution characterisation of *X. fastidiosa* strains. Across the analysed samples, *X. fastidiosa* was detected in multiple hosts and environments, with strains assigned to several subspecies and new *X. fastidiosa*-host associations being revealed. Phylogenetic and typing analyses revealed previously undescribed allelic variants and novel sequence types within established subspecies, indicating ongoing diversification rather than the emergence of highly divergent lineages. By integrating complementary surveillance infrastructures with advanced molecular diagnostics, this approach directly supports EU priorities on early detection, improved epidemiological understanding, and identification of *X. fastidiosa* emerging variants. The combined use of sentinel plots and botanic gardens strengthens the capacity to detect, validate, and interpret emerging *X. fastidiosa* diversity, informing risk assessment and supporting sustainable management strategies.

P131. Distribution, colonisation and detection of *Xylella fastidiosa* in important plant hosts for the UK

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Sampling different host plants for diagnostics of *Xylella fastidiosa* should be informed by knowledge of the distribution and colonisation of the bacteria in plants in different conditions. The aims of our studies are to understand spread, latency and symptom development in key high-risk plant species for the UK. High risk ornamentals and trees were artificially inoculated with different subspecies of *Xylella fastidiosa* in glasshouse conditions, symptoms were observed, and the plants were sampled and tested by qPCR. In the first experiment, three host species (lavender, oleander, coffee) were inoculated with three subspecies of *X. fastidiosa* and kept in two different glasshouse environments (25°C and ambient temperature) where rates of colonisation and symptom expression were monitored. Symptoms that could be due to *Xylella* infections were not always associated with positive qPCR samples; oleander and coffee plants were successfully infected and, although most positive samples were close to inoculation points, some oleander samples taken up to 10cm away from the inoculation points were also positive showing that the infection had progressed. In the second experiment, infection of seven plant species considered high risk for the UK (coffee, rosemary, blueberry, grapevine, bay laurel, oleander and plum) with three subspecies of *Xylella*, at a range of temperatures, showed that *Xylella* can infect these plant species in typical UK spring to autumn temperatures (from 10 - 25°C), but that bacteria move slowly, thereby increasing the time for detection of the bacterium. In a third experiment, inoculations of four tree species (oak, elm, willow and *prunus*) with three subspecies resulted in successful infections of approximately ¾ of oak, ½ of elm and *prunus* trees and only one willow tree, whilst all oleander control plants tested positive. A fourth experiment is now running with oak trees kept at different temperatures. Our results generally showed different success rates of infection between the species tested with some differences between the isolates used and the glasshouse temperature conditions; detection of *Xylella* was more successful in samples that were collected close to the point of infection and therefore, the plants should be sampled at multiple points and times to maximise the chance of *X. fastidiosa* detection.

P132. Survey of *Xylella fastidiosa* in North Macedonia

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Xylella fastidiosa is a quarantine plant pathogenic bacterium of major economic importance. In the Republic of North Macedonia, surveys for *X. fastidiosa* have been conducted since 2016 as part of the national plant health survey programme coordinated by the Phytosanitary Directorate, the National Plant Protection Organization (NPPO). The survey is designed using a risk-based approach. Survey focuses on high-risk locations such as nurseries, garden centres, border control points and major transport routes. Surveys are carried out by the State Phytosanitary Inspectorate and authorized scientific institutions in accordance with EU standards and EFSA guidelines. In 2025, a total of 400 visual inspections were conducted and 140 samples were collected and analysed using molecular diagnostic methods. All samples tested negative for *X. fastidiosa*. The survey results confirm that *X. fastidiosa* is not known to be present in the Republic of North Macedonia. Continued surveillance and preventive phytosanitary measures remain essential to prevent the introduction and establishment of this harmful pathogen.

P133. Monitoring and detection of *Xylella fastidiosa* in the Latium region

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Phy.Dia. Srl. is a laboratory accredited by the Latium Region for the monitoring and detection of *Xylella fastidiosa* (*Xf*) within the regional monitoring program conducted in Latium during the five-year period 2021–2025. In this framework, Phy.Dia Srl. collaborates with the Phytosanitary Regional Service (PRS) to ensure continuous territorial surveillance and, when necessary, the prompt activation of containment measures for *Xf* outbreaks. Detection of *Xf* was performed in accordance with EPPO standard PM7/24 (5) – Appendix 5 using the Harper method, by qualified technical personnel. The monitored species included *Olea europaea*, *Prunus dulcis, Lavandula spp.*, *Salvia spp.*, *Laurus nobilis*, *Nerium oleander*, *Rhamnus alaternus*, and *Acacia spp*. Since 2021, Phy.Dia Srl has analyzed more than 9,200 potential host plants sampled across the regional territory. During the five years of monitoring activities, two *Xf* outbreaks were identified. The most affected species was *Prunus dulcis*, a rapidly expanding crop in the Latium region. In all positive host plants, the detected subspecies was *Xylella fastidiosa* subsp. *multiplex*. These findings highlight the strategic importance of maintaining an efficient monitoring and early-detection system for quarantine plant pathogens such as *Xf*, particularly in the context of the ongoing expansion of almond cultivation in the region.

P134. Satellite-Based Monitoring Services for the Management of *Xylella fastidiosa*: A Strategic and Business Model Analysis

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Recent advances in Earth Observation technologies and the increased availability of satellite imagery have expanded the possible applications of remote sensing in agriculture. Despite these advancements, the development of operational services for plant disease monitoring remains limited, especially for *Xylella fastidiosa*, a pathogen that causes important economic and environmental damage in Mediterranean olive-growing regions. This study introduces the FIXYLL project (FIXYLL FIght XYLeLla fastidiosa - DTA - Distretto Tecnologico Aerospaziale), which assesses the potential of satellite-based monitoring services to support the containment and management of *Xylella fastidiosa*. The analysis inspects four proposed services: identification of vigorous olive trees within heavily infected areas, spatial-temporal visualization of disease dynamics, verification of compliance with phytosanitary soil management practices, and monitoring and validation of tree removal activities. Some of these services primarily support research and communication, whereas others demonstrate greater potential for operational deployment within phytosanitary monitoring and control systems. The research uses a case study approach centered on the Apulian olive sector. Data collection included expert interviews with key informants from scientific, technological, and institutional backgrounds, supplemented by literature and project documentation. A SWOT analysis assessed internal and external factors influencing the development of the proposed services. The strategic configuration of these services was further analyzed using the Business Model Canvas framework to identify key partners, customer segments, value propositions, and revenue mechanisms. The results show both benefits and implementation barriers for satellite-based monitoring services in agriculture. Specifically, services focused on compliance monitoring and verification of phytosanitary measures are closer to market deployment, whereas others require additional technological and methodological development. The study also stresses the importance of stakeholder participation, indicating that farmers could act as “prosumers” by providing field-level data essential for the effective operation of remote sensing monitoring systems.

P135. Detection of symptoms induced by pierce’s disease with high-resolution hyperspectral and thermal imagery

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Pierce’s disease (PD), caused by the bacterium *Xylella fastidiosa* (*Xf*), is one of the most devastating diseases affecting grapevines worldwide. Its detection in Mallorca in May 2017 highlighted the threat posed by PD to European viticulture. Early detection of *Xf* infections is essential for effective prevention and management of PD. Remote sensing (RS) approaches have shown strong potential for early detection of *Xf*-induced symptoms by identifying distinct spectral traits associated with *Xf* in olive and almond. This study aims to identify such traits in grapevines affected by PD, contributing to a generalised RS-based framework for *Xf* detection and facilitating early, large-scale disease surveillance. We investigated RS plant traits including pigment content, canopy structure, solar-induced fluorescence (SIF@760), the thermal-based Crop Water Stress Index (CWSI), and narrow-band hyperspectral indices derived from high-resolution imagery to determine those most responsive to *Xf* infection and associated physiological damage in grapevine. Two extensive field campaigns were conducted in *Xf*-affected vineyards on Majorca Island (Balearic Islands, Spain) in 2023 and 2024, covering 13,571 and 7,477 vines respectively. Visual inspections were performed to assess PD severity, with qPCR testing for *Xf* in at least 10% of plants, including asymptomatic individuals, alongside airborne campaigns acquiring high-resolution thermal and hyperspectral imagery across the visible-near-infrared (400-850 nm) and shortwave infrared regions (950-1700 nm). A multistage machine learning (ML) approach was applied to detect and discriminate *Xf*-induced spectral changes, identifying traits specific to PD. Results indicate that NPQI, the xanthophyll proxy PRIn, SIF@760, and CWSI are among the most responsive spectral plant-trait indicators for detecting *Xf*-induced physiological changes in grapevine. Incorporating these traits into the ML approach yielded promising classification performance, with overall accuracy exceeding 70%. The most sensitive spectral plant-trait indicators for detecting *Xf*-induced changes in grapevine will be discussed, emphasising their differences from traits observed in olive and almond, and highlighting their potential use for operational large-scale surveillance in regions at risk of *Xf* infection.

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P136. Early physiological signals of Olive Quick Decline Syndrome detected through satellite-based time series analysis

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Xylella fastidiosa (*Xf*) is one of the most dangerous plant pathogens worldwide, due to its broad host range and severe socioeconomic and environmental impacts. Early detection and continuous monitoring of its spatio-temporal dynamics are crucial to limiting its spread and mitigating its impact. Satellite-based remote sensing represents a promising tool for consistent, large-scale monitoring of *Xf* outbreaks. However, previous efforts based on single-date or single-season multispectral imagery have yielded limited accuracy in detecting early stages of infection, likely due to insufficient spectral, spatial, and temporal resolution. In this study, e investigated whether satellite time series can reveal pre-symptomatic signals of vulnerability in olive orchards that later develop OQDS. We focused on a well-documented outbreak hotspot in Apulia (southern Italy) and analysed PlanetScope imagery (3m pixel size) over four years (2017–2020), using 2020 as the year of confirmed infection. We derived Soil Adjusted Vegetation Index (SAVI) time series from cloud-free scenes, generated 8-day maximum value composites, interpolated missing observations, and applied Savitzky–Golay smoothing to obtain consistent seasonal profiles. We then compared temporal behaviour of healthy and infected trees using interannual correlation analysis, principal component analysis of multi-annual SAVI trajectories, and Dynamic Time Warping to quantify profile divergence across years. Results show that trees classified as infected in 2020 exhibited consistent pre-infection alterations in seasonal SAVI dynamics compared with healthy trees, including lower interannual similarity and distinctive trajectory shifts. These findings indicate that multi-temporal satellite indicators can capture subtle physiological changes before clear symptoms become visible, suggesting a strong potential for early detection at large-scale through the analysis of time-series of multispectral satellite imagery.

P137. Integrating proximal sensing and plant physiology to monitor water status in super-intensive olive orchards: a multi-site study on *Xylella*-resistant cultivars

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This study evaluates the physiological and spectral response of olive trees across two experimental sites in the Apulia region (Italy). The research focuses on the behavior of *Xylella*-resistant cultivars within super-intensive planting systems, specifically investigating the Favolosa (FS17) cultivar in Site 1 (tree age 6 years) and Site 2 (tree age 3 years). Three irrigation regimes were tested: well irrigated (WI), moderate-deficit irrigated (MDI) and severe-deficit irrigated (SDI). The core objective was to assess the correlation between indicators, such as Stem Water Potential (SWP) and Stomatal Conductance (gs), morphometric measurements and multispectral Vegetation Indices (VIs) derived from proximal sensing. Physiological measurements provided a robust validation of the stress gradient. SWP showed a clear separation between treatments; while the WI group remained within a functional range (-1.5 to -2.5 MPa), the SDI group frequently dropped below -3.5 MPa, reaching critical peaks of -6.0 MPa at Site 1 during the August-September period. This hydraulic strain was mirrored by gs values, which exhibited a near-total metabolic shutdown in SDI trees during peak summer months, significantly limiting gas exchange compared to the more resilient MDI group. A key methodological feature was the calculation of VIs, which successfully isolated the irrigation effect from site-specific variables like soil texture and tree age. The results demonstrate that water stress induces a systemic change in leaf chemistry rather than just a loss of biomass. While structural indices like NDVI remained relatively stable due to the sclerophyllous nature of olive leaves, efficiency-based indices showed high sensitivity. The Photochemical Reflectance Index cluster (PRI), Anthocyanin reflectance index (ARI), pigments carotenoids and chlorophyll clusters and the water stress/content based clusters emerged as superior "early warning" indicators displaying a progressive spectral modulation. These findings suggest that by monitoring specific spectral clusters, particularly those sensitive to the xanthophyll cycle and chlorophyll density, it is possible to implement highly targeted irrigation strategies, optimizing water use in super-intensive systems without reaching critical thresholds of permanent physiological damage.

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P138. A portable qPCR diagnostic workflow for reliable on-site detection of *Xylella fastidiosa* in major mediterranean crops

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Since its introduction in Apulia in 2013, *Xylella fastidiosa* subsp. *pauca* caused an agronomic, economic, and ecological decline. As no effective curative treatments are available, the management of *X. fastidiosa* relies on prevention, early detection and strict phytosanitary measures. Surveillance programmes are crucial for timely identification and eradication of new infection outbreaks, representing an essential component of preventive phytosanitary defence. To date, detection and quantification of *X. fastidiosa* are based on molecular diagnostic methods, however, standard workflows rely on laboratory-based DNA extraction protocols, requiring the transport of samples from field to specialized laboratories. The recent availability of portable molecular platforms opened new opportunities for on-site detection. Here, we describe a rapid and accurate workflow for on-site detection and quantification of *X. fastidiosa* ssp. *fastidiosa*, *multiplex*, and *pauca* in grapevine, almond, and olive, respectively, integrating a rapid field-safe DNA extraction method with qPCR in portable thermocycler. A 30 minute safe extraction protocol, adapted for field with portable instrument is proposed. Plant tissues were macerated in lysis buffer (2% CTAB, 4% PVP, 1.4 M NaCl, 100 mM Tris-HCl pH 8.0, 20mM EDTA) at 65°C for 10 min. Following the addition of 5M potassium acetate, samples were incubated on ice and centrifuged at 6,000 rpm for 10 min. DNA was precipitated with isopropanol by 10 min centrifugation and resuspended in ultrapure water. qPCR was conducted using primer pair already described (Harper et al., 2010) in a portable thermocycler. gDNA yields and quality comparable to official EPPO method were obtained. The analytical sensitivity of qPCR assay was evaluated on olive, almond and vine samples, inoculated with bacterial suspension (102 to 107 CFU/mL) of *X. fastidiosa* subsp. *pauca*, multiplex and fastidiosa, respectively. The limit of detection and quantification were $1 \cdot 10^2$ CFU/mL, corresponding to 36±0.2, 35±0.0 and 38±0.3 cycles, for olive, almond and grapevine, respectively. The workflow was successfully validated on naturally infected olive samples in compliance with EPPO standard (PM7/24 (4)—Annex 5). Reducing diagnostic turnaround time and minimizing the risk of pathogen spread associated with sample transport, this workflow enables a rapid and accurate on-site detection of *X. fastidiosa* in compliance with EPPO sensitivity, specificity and reliability requirements

P139. Combining metabolomics and proximal sensing measurements for early detection of *Xylella fastidiosa* infection in olive plants

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Xylella fastidiosa (*Xf*) is a Gram-negative bacterium that colonizes the xylem vessels of several plant species. The extreme severity of the desiccation symptoms in olive led to the description of olive quick decline syndrome. Previous electron microscope studies showed that *Xf* exploits and degrades the pit membranes of olive susceptible cultivars to spread systemically. Occluded vessels of susceptible cultivars contained an amorphous electrondense matrix resembling gum, while in resistant cultivars occluded vessels were mainly filled by callose-like granules that tightly entrapped *Xf* cells. Within the REACH-XY project, we aim to use targeted and untargeted metabolomics (HPLC-DAD-Q-ToF) approaches to investigate the compounds, observed using transmission electron microscopy (TEM), in the xylem vessels of olive trees after *Xf* infection. These approaches will help to identify specific biochemical markers that can discriminate healthy and infected plants and elucidate the resistance mechanism to *Xf* infection in susceptible and resistant olive cultivars. In addition, we aim to test if the *Xf* infection induces specific leaf spectral signature detectable by proximal sensing measurements, to integrate these data with metabolomics results. The measurements and collection of plant material were performed in two different experiments: 1) one greenhouse experiment using one-year-old plants from three cultivars characterized by different susceptibility to *Xf* (Arbequina, Leccino and Lecciana); 2) one field experiment in an olive orchard in Squinzano (Apulia region), hosting both infected and not infected trees of Fs17 cultivar. Branch samples were collected and split into two portions: one was used to analyse callose deposition by TEM, and the other one was frozen in liquid nitrogen for HPLC-DAD-Q-ToF analysis, after bark removal. For proximal sensing measurements, leaves were scanned with a portable spectroradiometer equipped with a contact probe, to obtain leaf reflectance indices that can be correlated with physiological parameters and metabolic markers. By identifying metabolites associated with xylem vessel depositions in susceptible and resistant cultivars of *Xylella fastidiosa*, these analyses provide insight into the biochemical mechanisms underlying plant responses to the pathogen. In parallel, proximal sensing data will be analysed to detect infection-related spectral signatures, enabling the development of tools for early detection.

P140. Detection of *Xylella fastidiosa* in spiked dormant plant material (almond and grapevine) and in resprouting olive plants

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Following the outbreak of OQDS caused by *X. fastidiosa* subsp. *pauca* in Apulia, *X. fastidiosa* subsp. *fastidiosa* was also detected in grapevine in the region, and subsp. *multiplex* in almond and other plant species in Apulia, Tuscany, and Latium. In this context this study evaluated different aspects: i) influence of different plant matrices available across different seasons on dormant plant of grape and almond for the detection and identification of *Xf*; ii) fitness of *Xf* in resprouting olive plants located in the infected area. To address the first objective the analytical sensitivity of four molecular diagnostic assays—real-time PCR (Harper), digital-droplet PCR (ddPCR-Dupas), tetraplex real-time PCR (Dupas), and real-time PCR (Hodgetts)—for the detection and subspecies identification of *Xff* in grapevine and *Xfm* in almond were evaluated. Samples were collected at four seasonal time points and artificially spiked. Among the detection tests, ddPCR resulted the most sensitive assay, consistently detecting up to 10^2 CFU/g of plant tissue across matrices and sampling periods, thus making it particularly suitable for the surveillance of hosts with low bacterial concentrations. Regarding *Xf* identification, real-time PCR Hodgetts demonstrated greater sensitivity compared to Dupas. The second aim of this work was related to the recent observation of canopy resprouting in olive trees in the infected Salento area (Italy). The seasonal dynamics of *Xfp* were monitored (2023–2025) in olive orchards showing high symptomatic (HS) index compared to olive groves with low symptomatic (LS) index due to a spontaneous canopy restoration. The vegetation index analysis (NDVI, SAVI, NDWI) showed a general decline for all assessed indices in both LS and HS group with a clearly larger magnitude of reduction in HS with respect to LS thus confirming a different behaviour among LS and HS orchards. However, no statistically significant differences were observed in bacterial loads across the different sampling periods in both HS and LS orchards. The incidence of the vectors and the *Xf* load in the insects were also evaluated, but no differences were detected between LS and HS orchards. Among *P. spumarius* and *N. campestris*, this latter was clearly dominating, making up 86% of total adults collected. In conclusion, the findings highlight the need for further research on the olive resprouting phenomenon, its long-term stability, and its potential links to additional factors

P141. Early detection of *Xylella fastidiosa*: portable diagnostic approaches using Lab on Chip and Oxford Nanopore Sequencing

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The spread of plant pathogens represents a major challenge for phytosanitary surveillance. Rapid detection of *Xylella fastidiosa* (*Xf*), a quarantine bacterium responsible for severe outbreaks in Mediterranean crops, is crucial to limit disease spread. Therefore, rapid, sensitive and portable diagnostic tools are essential for pathogen detection from plant material. In this context, two approaches are presented: Lab-on-Chip (LoC) technology and Oxford Nanopore sequencing for *Xf* diagnosis. Compared with conventional methods, LoC systems offer: compactness enabling onsite analysis, high surface-to-volume ratios enabling faster heat transfer and reduced assay time, and low reaction volumes with reduced reagent consumption. In this work, a LoC for *Xf* diagnosis by real-time PCR was assessed. The LoC consists of a system-on-glass (SoG) integrating a heater element, temperature sensors and fluorescence detection based on thin film optoelectronic. The SoG is optically aligned with a glass-PDMS microwell plate for sample handling. The device is housed in a metallic box ensuring portability. The LoC was tested for real-time PCR of *Xf* following Harper et al. (2010). *Xf* spiked samples of olive and grapevine leaves, as well as *Philaenus spumarius*, were tested. Preliminary results showed a limit of detection down to 10^1 CFU/mL with faster analysis compared with a standard thermocycler. In parallel, a Nanopore-based workflow for *Xf* diagnosis was developed using the insect vector. This work extends a previously developed amplicon-sequencing strategy targeting seven housekeeping genes, which demonstrated detection and identification at subspecies and sequence type (ST) level on infected plant samples. The workflow was therefore evaluated on *Philaenus spumarius* for surveillance. Preliminary results indicate that the pathogen can be detected at subspecies level down to 10^1 CFU/mL. However, the glt gene was not consistently recovered, suggesting the need for further optimization for ST identification. A shotgun metagenomic approach based on direct sequencing of total DNA extracted from spiked olive samples (*Xf* subsp. *pauca*) was also investigated to bypass gene amplification. Initial tests using Flongle flow cells with rapid library preparation showed encouraging results at high bacterial concentrations. Ongoing work is evaluating ligation-based library preparation with standard MinION flow cells to increase sequencing depth and improve sensitivity.

P142. Enhancing *Xylella fastidiosa* detection: integration of smart portable iot electronic noses with ptr-tof-ms

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The management of *Xylella fastidiosa* is a significant challenge for Mediterranean agricultural safety. It is essential to optimize diagnostic protocols that combine rapid detection capabilities with non-invasive sampling techniques to ensure cost-effective pathogen inspection. This study, funded by MASAF (DIACOX project, D.M. 664531/2022), explores the effectiveness of a portable smart IoT digital electronic nose for the early detection of the pathogen through the characterization of its Volatile Organic Compounds (VOCs). The research integrates Artificial Intelligence (AI) to identify the “olfactory fingerprint” of the bacteria across different substrates, in particular, bacterial cultures and plant samples. To validate the non-specific signals of the electronic nose, high-resolution Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS) was employed as support analysis, enabling the precise identification of the chemical constituents of the bacterium's volatilome from bacterial cultures of *X. fastidiosa*. Results from PTR-ToF-MS highlighted significant mass peaks corresponding to specific compounds that discriminate the bacterial cultures. Data processing was performed using Partial Least Squares Discriminant Analysis (PLSDA) to construct robust classification models. For the electronic nose, the models achieved a good correct classification rate in distinguishing infected from healthy samples across all tested culture media and plant materials. Variable Importance in Projection (VIP) scores consistently identified specific sensor channels as major contributors to the diagnostic output, demonstrating the stability of the bacterial signal across different samples. Similarly, PTR-ToF-MS models reached adequate accuracy, correlating the electronic nose digital patterns with specific VOCs characterizing *X. fastidiosa*. These findings suggest that integrating digital olfactory technology and appropriate modelling provides a promising approach for the development of ‘early detection’ systems. Despite environmental sensitivities, the alignment of electronic nose fingerprints with mass spectrometry validation highlights the potential for utilizing portable, real-time monitoring devices.

P143. Optimized protocol for high-molecular-weight DNA extraction from olive nuclei

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Within the framework of the NOVIXGEN project, the resequencing of different clones of the olive cultivar Leccino, known for its tolerance to *Xylella fastidiosa*, required the isolation of high-molecular-weight (HMW) DNA suitable for long-read sequencing and advanced genomic analyses. However, obtaining intact DNA from woody perennial species remains challenging due to the abundance of secondary metabolites and polysaccharides that interfere with DNA isolation procedures. In olive (*Olea europaea L*.), these biochemical characteristics often result in DNA degradation or low yields when standard extraction protocols are applied. An optimized protocol for the extraction of HMW DNA from olive nuclei was developed, starting from the method previously described. Several modifications were introduced to improve nuclei purification and DNA integrity while maintaining high recovery yields. The procedure includes an enhanced nuclei isolation workflow based on sequential washing steps combined with repeated filtration through Miracloth to efficiently remove cellular debris and contaminants. The protocol also integrates additional purification steps and a detergent-based treatment to improve nuclei cleaning before lysis. Nuclei lysis was performed using a buffer containing SDS, EDTA and proteinase K, followed by phenol/chloroform purification and ethanol precipitation to recover intact genomic DNA. Key optimizations included stricter filtration procedures, multiple washing steps to increase nuclei purity, and modifications in the handling and drying of DNA pellets to minimize shearing and degradation. These adjustments proved critical for preserving DNA integrity and improving overall yield. The optimized protocol allows the recovery of abundant HMW DNA suitable for downstream genomic applications, including long-read sequencing and structural genome analyses from olive samples.

P144. Preliminary assessment of a lamp-coupled crispr cas12a assay for *Xylella fastidiosa* detection

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Accurate and reliable diagnostic tools are crucial to limit the spread of *Xylella fastidiosa*. To develop a specific and potentially portable CRISPR/Cas12 based assay, we optimized a LAMP reaction using primers and probes targeting the rimM gene (Harper et al., 2010). To avoid contamination two strategies were tested: LAMP with dUTP and Antarctic Thermolabile UDG, and WarmStart LAMP Master Mix with UDG (NEB). Assays were optimized using purified bacterial DNA, evaluating commercial kits, reaction conditions, and dUTP concentrations with UDG at different incubation temperatures, comparing reactions with and without UDG. WarmStart LAMP Master Mix with UDG, with a 10min pre incubation at 25°C followed by 65°C for 30 min, yielded the lowest carryover contamination and best repeatability. This optimized LAMP was then coupled to a CRISPR Cas12 fluorescent detection system in two and one step formats. Specific rimM targeting gRNAs and a fluorescent reporter (Farral et al., 2024) were tested with different Cas12 concentrations and incubation times. The most efficient detection used 50 mM Cas12 Ultra, 62.5 nM gRNA, and 750 mM reporter, with incubation up to 90 min at 37°C. Analytical sensitivity was preliminarily assessed using 10 fold dilutions of purified rimM recombinant plasmid DNA (0.5 nM–0.5 aM) and DNA from artificially contaminated olive sap spiked with strain De Donno subsp. pauca ST53 (10^6–10 CFU/ml). In standalone LAMP, maximum fluorescence (~800k) was detected across plasmid DNA and contaminated samples within 9–16 min. As template concentration decreased, LAMP continued to generate high signals, with LOD at 0.5 fM for plasmid DNA and ~10 CFU/ml for contaminated sap at ~16 min, though detection became inconsistent at lower levels. In LAMP–CRISPR Cas12 assays, the two-step system showed a detection limit of 0.5 fM at ~12 min. The cap drop one step assay displayed lower sensitivity, with an LOD of ~5 pM and fluorescence of ~1.5k after 60 min. Based on these results, preliminary tests were initiated to improve the sensitivity of the single reaction format and to evaluate both assays on purified DNA and crude alkaline sap from olive samples, aiming to eliminate DNA purification and accelerate field deployable diagnostics.

P145. Optimization of *Xylella fastidiosa* Detection in Olive Trees Using Membrane-Based DNA Extraction Coupled with LAMP Assay

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Early and accurate detection of plant pathogens is critical for managing harmful organisms when they emerge in new areas, such as Olive Quick Decline Syndrome (OQDS) in olive trees caused by *Xylella fastidiosa*. An improved real-time loop-mediated isothermal amplification (LAMP) protocol was developed using pathogen imprints printed on nitrocellulose membranes, providing a rapid detection method for this bacterium. Naturally infected olive stem imprints on nitrocellulose membranes were stored at room temperature and tested after extraction by real-time LAMP over the time. These samples were compared with extracts obtained from conventional plant material stored in standard conditions. The performance of the LAMP assay combined with the membrane-based extraction method was evaluated in parallel with a reference qPCR assay. The membrane-based extraction method maintained high detection accuracy while enhancing biosafety, simplifying sample handling, and reducing processing time. Furthermore, the real-time LAMP assay achieved 100% diagnostic specificity and high sensitivity, with a detection limit of 10^2 cfu/ml. In conclusion, the integration of this low-resource and storage-friendly membrane-based extraction method expands the applicability of LAMP for routine detection of *X. fastidiosa* in field surveys, nurseries, and phytosanitary entry points, supporting surveillance programs targeting quarantine pathogens.

P146. Comparison of Culture Media and qPCR-Based Kits for the Detection of *Xylella fastidiosa*

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Xylella fastidiosa (Wells et al.) is a phytopathogenic bacterium responsible for significant economic losses in numerous plant species, particularly in countries across the Americas, Asia, and Europe. In Azerbaijan, it is included in the A1 quarantine list, while within the EPPO it is classified under the A2 quarantine list. The pathogen is characterized by a wide host range and induces symptoms such as leaf scorch, wilting, chlorosis, marginal necrosis, and general plant decline. In severe cases, infection may result in plant death. The rapid dissemination potential and capacity of *X. fastidiosa* to establish latent infections necessitate early and accurate diagnosis for effective phytosanitary control. The aim of this study was to determine the most suitable culture medium for the growth of X. fastidiosa in accordance with the EPPO PM 7/024 protocol, using different nutrient media (BCYE, PWG, and PD2), and to comparatively evaluate the analytical sensitivity and specificity of different real-time PCR kits for its identification. For this purpose, detection and identification methods of *X. fastidiosa* were investigated between 2024-2026 at the Central Phytosanitary Laboratory of the Azerbaijan Food Safety Institute. Examinations were conducted on samples belonging to 86 host and non-host plant species submitted to the laboratory through import inspections and national monitoring programs. No detection of *X. fastidiosa* was recorded in these samples. Therefore, comparative evaluation was performed using certified reference material (*X. fastidiosa* DSM 10026 – active culture). The strain was prepared in seven serial dilutions (10^0–10^{-6}) and isolated on three different culture media (BCYE, PWG, and PD2). Real-time PCR analysis was performed comparatively using the Norgen Biotek PITM67000L-1 and the Dosabio *X. fastidiosa* qPCR Detection Kit (Cat. No. PB22-180). Ct values, analytical sensitivity, and specificity were evaluated. According to the results, the applied culture media demonstrated differential effects on the growth performance of *X. fastidiosa*. More intensive colony formation and active growth were observed on BCYE and PWG media, whereas comparatively weaker growth was recorded on PD2 medium. Real-time PCR analyses demonstrated high analytical sensitivity and specificity across all dilutions, with only minor variations in Ct values between the commercial kits.

P147. One way to discover the hidden life of *Xylella*

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Rapid detection of a pathogen in a new environment is crucial to preventing or limiting epidemics. However, this task can be extremely difficult, particularly for bacteria, which can have long latency periods and thus go undetected for long periods of time, and which have a wide range of hosts. Rather than resorting to new experiments, we have been exploring the possibility of using the largest public database of next-generation sequencing raw data, the Sequence Read Archive. This represents a unique opportunity to detect the presence of a given organism in sequencing data from various samples from around the world, obtained for research purposes unrelated to our study. The challenge of this work was to analyze this database in search of datasets showing signatures of *Xylella* in order to potentially discover new aspects of the ecology of this bacterium. The NCBI tool, SRA Taxonomic Analysis Tool, identified 8,564 datasets with at least 1,000 *Xylella* reads. Searching for eight *Xylella*-specific signature sequences reduced the number of potentially infected datasets to 704. Of these, 605 involved sequencing projects of *Xylella* strains or known *Xylella* hosts. We confirmed the presence of *Xylella* sequences in 62 of the remaining 97 datasets. Among these 62 datasets, we were able to identify i) two new strains of *X. fastidiosa* subsp. *multiplex* in the USA, one representing a new Sequence-Type and the other from a plant species that had never before been reported as a host of X. fastidiosa; ii) two new strains of *X. fastidiosa* subsp. *pauca* from Coffea spp. in Brazil and Colombia; iii) the potential presence of Xylella in two new countries, Nicaragua and Namibia; iv) a new lineage in the species *X. taiwanensis* in Japan; v) a new species of *Xylella* detected in a wild rice species sampled in China and Myanmar. In this latter case, four genomic sequences were assembled and one of them was circularized. It represents a new species that we propose to name *X. rufipogonis*. This species has a unique repertoire of secretion systems, including, in particular, a chromosomal T4SS. In conclusion, this work highlighted the existence of a novel *Xylella* species, *X. rufipogonis*, that shall now be pursued by the isolation of strains and their further characterization. As a more general perspective, this approach could be implemented for other regulated organisms in order to expand knowledge about their genetic diversity, spatial distribution, and reservoirs.

P148. Detection and subspecies identification of *Xylella fastidiosa* in host plants and insect vectors by nanoplate digital PCR

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Droplet digital PCR (ddPCR) provides high precision and sensitivity for low abundance targets but requires dedicated droplet generation consumables and is relatively time consuming. Nanoplate dPCR, by contrast, partitions samples into fixed microchambers without oil emulsions, enabling a faster and automation friendly workflow suitable for routine diagnostics. In this study, using purified DNA samples, we standardized and validated two nanoplate dPCR protocols based on (i) the Harper et al. (2010) primers/probe set for *Xylella fastidiosa* detection—combined with internal controls, either COX or 18S, and (ii) Dupas et al. (2019) for the simultaneous identification of subspecies *pauca*, *fastidiosa* and *multiplex*. Sensitivity and accuracy of the Harper based assay were assessed by testing five plant matrices (*Olea europaea*, *Vitis vinifera*, *Prunus dulcis*, *P. avium*, *Polygala myrtifolia*) and the insect vector Philaenus spumarius, artificially contaminated with 10 fold serial dilutions (from 10^6 to 10 CFU/ml) of a bacterial suspension of strain De Donno subsp. *pauca* ST53. Two nanoplate formats were compared: the 26K 24 well plate (26,000 partitions/well) and the 8.5K 96 well plate (8,500 partitions/well). Both showed high efficiency, with R^2 values of 0.9876–0.9999 for the 8.5K format and 0.9975–0.9999 for the 26K format. The 26K plates provided the lowest detection limits, reaching 10 CFU/ml in *V. vinifera* and *P. avium*, and 10^2 CFU/ml in the remaining matrices. With the 8.5K format, the limit of detection was 10^2 CFU/ml for all matrices except *P. myrtifolia*, which showed 10^3 CFU/ml. The subspecies specific nanoplate dPCR assay was validated on DNA purified from P. dulcis sap preparations simultaneously spiked with subsp. *fastidiosa* (ST1), *multiplex* (ST26) and *pauca* (ST53), tested in triplicate across 10 fold dilutions from 10^6 to 10 CFU/ml. Using the 26K format, analytical sensitivity exceeded that of qPCR, with a detection limit of 10 CFU/ml for all the subspecies with minimal variations in the number of replicates detected for the last dilution. Furthermore, in preliminary tests the subspecies specific assay successfully identified the target strains also in insect samples artificially contaminated. Finally, the diagnostic performance of the optimized protocols was validated on naturally infected plant and insect samples selected based on the results of qPCR reactions.

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P149. Early detection of *Xylella fastidiosa* infection using proximal sensors: identifying a common physiological response across olive, almond and grapevine

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Field diagnosis for *Xylella fastidiosa* (*Xf*) is often challenging; therefore, detecting infected plants before visible symptoms appear is essential for effective epidemiological surveillance and disease management. This study aimed to identify physiological and biochemical traits potentially associated with *Xf* infection using proximal sensors. Several *Xf* strains, representative from European outbreaks, were artificially inoculated into olive, almond and grapevine to monitor plant responses to *Xf* infection under controlled conditions. Additionally, naturally infected almond trees and grapevine orchards were selected in Mallorca Island and leaves from healthy and infected trees with asymptomatic or symptomatic branches were evaluated. Leaf measurements were performed using four proximal sensors: a Dualex sensor, a Licor porometer, and two PolyPen spectrophotometers covering the visible and near-infrared spectral regions (400–1000 nm). Measurements were collected from healthy and infected plants, and infection status was confirmed by qPCR analysis of petiole and stem samples. The analysis of 78 vegetation indices revealed clear spectral patterns that distinguished healthy plants from *Xf*-infected plants across the three host species. Linear discriminant analysis (LDA) selected between 11 and 15 vegetation indices, depending on the evaluation date and host species, with strong discriminatory capacity among plant health conditions. Across crops and evaluation dates, the resulting LDA models achieved classification accuracies above 78.6%, with Cohen's kappa values greater than 0.59 in all models. The indices contributing most strongly to discrimination of infected plants, including asymptomatic plants, were mainly associated with carotenoids, xanthophylls, chlorophyll fluorescence and stomatal conductance. These results indicate consistent physiological responses to *Xf* infection that can be detected through spectral traits across host species. Identifying common physiological indicators of infection could optimize surveillance strategies and improve the detection of asymptomatic infections without relying on host- or cultivar-specific physiological responses. Such early physiological indicators could also benefit breeding programs by enabling rapid screening of host responses to *Xf* in crops where disease symptoms develop slowly.

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P150. Climate suitability for *Xylella fastidiosa* and its subspecies in the EU: an updated multi-method assessment

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As part of the update of the EFSA pest risk assessment for *Xylella fastidiosa*, a new assessment of climate suitability was developed for the species and for the subspecies *fastidiosa*, *multiplex* and *pauca* across the EU territory. The analysis relied on an updated global occurrence dataset, harmonising records from the EFSA *Xylella* Host Plant Database, Member State surveillance (France, Italy, Portugal and Spain) and scientific and grey literature. Three complementary approaches were combined: a Köppen-Geiger climate-class screening, a bioclimatic threshold based on the bioclim variable bio06 (minimum temperature of the coldest month), and risk maps derived from the agreement of two species distribution models, an Ensemble-SDM and Bayesian Additive Regression Trees (BART). At species level, the Köppen-Geiger and bio06 approaches retained most of the EU as climatically suitable, with unsuitable areas confined to the coldest northern and high-mountain regions. Clear differences emerged among subspecies: *multiplex* showed the broadest climatic range (bio06 −15.9 °C), followed by fastidiosa (−3.1 °C), while *pauca* was the most restricted (3 °C). The Ensemble-SDM/BART maps retained more limited areas, concentrated in Mediterranean coastal and lowland regions. The two models often diverged, reflecting substantial uncertainty across much of the EU territory. Species-level outputs were largely driven by multiplex, supporting interpretation at subspecies level. Because climate is only one of the conditions required for establishment, the outputs should be read as evidence on its climatic component, to be interpreted together with information on host availability and vector presence.

P151. Eutopos: a novel topological, multidomain framework for ecological suitability modelling applied to *Xylella fastidiosa*

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Xylella fastidiosa poses a major threat to agriculture, and predicting its potential distribution requires robust assessment of environmental suitability across heterogeneous regions. Many presence-only modelling approaches are constrained by spatially biased occurrence data and often provide limited ecological interpretability, making it difficult to compare suitability patterns. In this context, we present EUTOPOS (European TOPOlogical Suitability), a methodological framework designed to evaluate the suitability for alien pests while ensuring ecological consistency and comparability across spatial scales and species. The framework has been applied to three *Xylella fastidiosa* subsps. to explore differences in their environmental suitability patterns in Europe. EUTOPOS is a presence-only multi-domain workflow combining PCA, clustering, and geometry-based environmental distances, discriminating among three suitability conditions (negative, marginal, core). Its modular design allows suitability to be assessed for individual or combined environmental domains (e.g. climate and land use), enabling transparent evaluation of how suitability evolve as new ecological information is incorporated. Application to *Xylella fastidiosa* subsps. highlighted substantial differences in their “eutopicity” patterns, demonstrating the ability of the framework to discriminate between closely related taxa. As for subsp. fastidiosa, core suitability areas were concentrated in Mediterranean countries, even if most of their territory was classified as marginal suitability. A preponderance of marginal suitability areas was also found in Central Europe, then declining towards negative suitability proceeding northern. Core suitability areas for subsp. *pauca* were limited and mainly concentrated along the Italian coastal zones, with marginal suitability extending across Mediterranean Europe, while most of the remaining European territory showed negative suitability. In contrast, subsp. *multiplex* showed a core suitability area much more extensive than the other two subsps., primarily concentrated in Southern Europe, with additional extensions into parts of Central and Eastern Europe. Within these regions, marginal suitability areas were also frequently observed. Conversely, Northern Europe was largely dominated by negative suitability. The identification of suitable areas offer insight on potential northward expansion gradients, fundamental for monitoring and surveillance efforts.

P152. Data collection and harmonised mapping of *Xylella fastidiosa* host plants in support of EFSA pest risk assessment

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Host plant distribution is a key component of pest risk assessment for *Xylella fastidiosa*, a regulated quarantine pathogen with a broad host range and major impacts on Mediterranean perennial crops. We developed a harmonised EU-wide data inventory and mapping workflow to support spatially explicit host distribution modelling for almond, citrus, olive and grapevine. The work addresses data collection and data integration and interoperability for crop mapping, including regional statistical data collection, compilation of EU-wide geospatial layers, use of auxiliary in-situ information, and alignment of all datasets to 10 × 10 km reference grid. A harmonised workflow was developed to integrate regional statistics, gridded agricultural census data, geospatial layers and auxiliary observations into a common 10 × 10 km reference grid. Reported crop information was used directly where available, while missing or suppressed values were estimated through weighted allocation based on high-resolution host-proxy layers and constrained by regional control totals. This approach enabled consistent EU-wide host mapping despite differences in data availability, thematic detail and spatial resolution between crops and countries. The resulting structured data archive and reproducible methodology provide a transparent basis for EU-scale host plant mapping in support of EFSA pest categorisation, pest risk assessment, survey design and scenario analysis for the potential spread and impact of *X. fastidiosa*. The approach also demonstrates how heterogeneous administrative, statistical and geospatial datasets can be integrated into interoperable crop-host layers for plant health applications.

P153. Towards a harmonized global occurrence dataset for *Xylella fastidiosa*: consolidating EFSA and complementary data for climate suitability modelling

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Correlative models for climate suitability analysis critically depend on the completeness and consistency of species distribution data. For *Xylella fastidiosa*, occurrence records are fragmented across multiple sources and are often used independently, limiting their effectiveness for large-scale modelling. This limitation is further amplified in analyses at finer taxonomic resolution, such as the subspecies level, which require additional filtering to retain only records with reliable subspecies identification. In this work, we present the development of a harmonized, global occurrence dataset for *X. fastidiosa* and the subspecies considered in the upcoming EFSA PRA (subsp. *pauca*, *fastidiosa* and *multiplex*), integrating heterogeneous data sources into a unified framework. Starting from the European Food Safety Authority *Xylella* Host Plant Database, we systematically incorporated additional records from EU Member States surveillance systems and expanded the dataset through targeted grey literature mining, including theses, phytosanitary bulletins, government alerts, technical reports, and non-English sources. A dedicated processing pipeline was implemented to extract, geo-reference and standardize occurrence data. This included parsing host plant records to derive spatial occurrences, converting administrative-level observations into geographic coordinates using geocoding approaches, and retrieving spatial information from georeferenced figures in scientific publications. The resulting dataset represents, to our knowledge, the most comprehensive and up-to-date global compilation of *X. fastidiosa* occurrences. By improving data completeness, spatial resolution, and accessibility, this resource provides a robust foundation for more reliable climate suitability assessments and epidemiological analyses.

P154. Landscape homogeneity and patch connectivity characterize olive orchards affected by *Xylella fastidiosa* in Apulia

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The spatial configuration of agricultural landscapes can influence the spread of plant pathogens by affecting host connectivity and vector movement. This study investigates whether landscape structure differs between olive orchards where *Xylella fastidiosa* has been recorded and the broader population of olive orchards in Apulia. Six landscape metrics, i.e., contagion (CONTAG), patch density (PD), landscape shape index (LSI), Shannon evenness index (SHEI), largest patch index (LPI), and mean patch area (AREA_MN), were compared between infected and background orchards. Non-parametric tests revealed significant differences for all metrics (Wilcoxon test, $p < 0.001$). Infected orchards were characterized by higher contagion and largest patch index values, indicating greater spatial aggregation and dominance of large olive patches, and by lower patch density, landscape shape complexity, and landscape evenness, reflecting reduced landscape heterogeneity. The Random Forest model identified Shannon evenness, contagion, and largest patch index as the most important predictors of pathogen presence. Principal component analysis confirmed a landscape gradient separating heterogeneous and fragmented landscapes from highly aggregated olive-dominated systems, with infected orchards positioned toward the latter. These results suggest that simplified and highly connected olive landscapes may facilitate the establishment or spread of *Xylella fastidiosa*. Incorporating landscape heterogeneity into agricultural planning may therefore contribute to improving the resilience of olive agroecosystems to emerging plant diseases.

P155. Multi-source IoT system prototype for the surveillance of *Xylella fastidiosa*

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Xylella fastidiosa (*Xf*), a quarantine plant pathogen responsible for Olive Quick Decline (OQD), represents one of the most serious threats to olive production worldwide. Early and reliable surveillance is essential to ensure rapid outbreak detection and the timely implementation of eradication or containment measures in accordance with current phytosanitary regulations. To address this challenge, a multi-source IoT-based surveillance prototype has been developed, integrating ICT, geomatics, diagnostic, and predictive tools. The system collects, harmonizes, and streams heterogeneous data to a dedicated platform, enabling real-time spatio-temporal monitoring of *Xf* infections and supporting data-driven decision-making for targeted control actions and optimized monitoring strategies. Early detection is implemented across multiple spatial scales. At large scale, multi-temporal analysis of Sentinel-2 imagery within the Copernicus Programme framework allows the identification of OQD-suspected olive groves through the detection of progressive biomass loss. At smaller scale, a UAV-based multispectral sensor prototype equipped with *Xf*-specific wavelength bands supports the early identification of infected trees before symptoms development. At field scale, monitoring activities are supported by the XylAppEU mobile application, which enables accurate data collection, storage, and real-time transmission. The system integrates multiple data sources, including satellite and UAV imagery to guide ground inspections, statistically robust sampling schemes for collecting plant material and "spy insects" across *Xf* demarcated areas, spy insect monitoring for pathogen testing in asymptomatic zones based on forecasting models, and a sentinel plant network deployed in high-risk locations to reveal early infections and/or attract vectors. Plant and insect samples are analyzed using a molecular real-time LAMP assay performed either directly in the field or under laboratory conditions. Furthermore, the use of printed nitrocellulose membrane disks for plant sampling, together with the collection of spy insects in sealed tubes, enables safe long-distance transport of samples while preventing pathogen spread. Although still at the prototype stage, the system has already demonstrated strong potential for guiding field inspections and enabling early detection of new infection foci.

P156. *Xylella fastidiosa* isolates from almond show differential biology in *Prunus* spp.

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In the Apulia region, almonds have been found infected by *Xylella fastidiosa* (*Xf*) isolates of *pauca* (*p*), *multiplex* (*m*) and *fastidiosa* (*f*), with infections recorded mainly in marginal agricultural areas, where disease impact appears so far negligible and no specific symptomatology associated. While field evidence has suggested resistant phenomena for *Xfp* ST53, almond turned to be the main host in the two newly discovered outbreaks in the region, where *Xff* and *Xfm* isolates have been characterized. To preserve almond-infected field sources (under eradication measures) for scientific aims, we have grafted *Xfm* and *Xff*-infected cuttings onto *Prunus* cerasifera seedlings and on a few potted plants of the almond cvs Avijor and Tuono and kept under quarantine facility. Symptom onset and bacterial spread have been regularly monitored in the scions and rootstocks. As expected, almost half of the top-grafted plants failed due to the poor quality of the material. In these failed grafts, no bacterium was detected below the grafting points, suggesting that without established vessel connections, mechanical transmission by contact of infected xylem tissues is not sufficient for bacterial invasion. Conversely, the bacterium was detected in the scions and/or rootstocks of the successfully grafted plants. At 12 months after grafting, samples taken from the shoots grown on myrobalan harbor high bacterial population (in 50% of plants) when *Xfm*-infected scions were top grafted, and very low bacterial titer when grafted with *Xff*-infected buds. Interestingly, none of the myrobalan shoots showed symptoms resembling those of leaf scorching, whereas leaves on the top-grafted cuttings exhibited the typical leaf scorching, more pronounced in *Xfm*-infected scions. The grafting performed on cvs Avijor and Tuono, although limited in numbers, showed that *Xff* was able to move downward and invade Tuono plants but not those of Avijor, while both were infected by *Xfm*. Comparative transcriptomic analyses to dissect specific bacterial-cultivar interactions are ongoing. Differentially expressed genes and gene ontology enrichment analyses have been completed, while the selection of candidate genes and their in vivo validation by qPCR are currently ongoing. Overall, these results indicate a different biology in *Prunus* spp. of the isolates belonging to the two subspecies, with *Prunus* spp. being more permissive to be colonized by *Xfm* isolates than *Xff*.

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P157. Introducing the project XYL-o-VINE: Ecology, ethology and transmission biology of *Xylella fastidiosa* by insect vectors in table grape vineyards

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(See thematic session 8.8b)

P158. Impact of *Xylella fastidiosa* subsp. *fastidiosa* infection on selected grapevine varieties relevant to Apulian viticulture

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In recent years, an emerging phytosanitary threat has increasingly affected Apulia, targeting one of the most strategic sectors of regional and national agriculture: table grape production. In 2024, *Xylella fastidiosa* subsp. *fastidiosa*, the causal agent of Pierce’s disease (PD) of grapevine, was detected for the first time in the municipalities of Triggiano and Noicattaro (Province of Bari), within one of Italy’s main table grape districts. The presence of the bacterium in such a highly specialized production area is epidemiologically significant, given the susceptibility of *Vitis vinifera* and the potential consequences in terms of vine decline, yield losses, and economic sustainability. Within the NOVIXGEN project, an experimental assessment was conducted to evaluate the response of V. vinifera varieties cultivated in Apulia to infection by *X. fastidiosa* subsp. *fastidiosa*. As the pathogen is currently not established in the region rather in the process of being eradicated, experiments were carried out in the Balearic Islands, which are infected zones, at the Consejería de Agricultura, Pesca y Medio Natural (Palma). Trials were performed over two consecutive years (2024–2025). A total of 12 cultivars were tested, including 8 wine and 4 table grape varieties. In 2024, plants were inoculated with strains XYL 2055/17 and XYL 2177/18; in 2025, strains XYL 1734 and V4 were used. All cultivars proved susceptible, though with variable symptom severity and bacterial colonization. Symptoms were consistent with PD and included marginal leaf necrosis, lamina abscission with petiole retention, incomplete shoot lignification with green islands, reduced vigor, and berry shrivelling. Among wine cultivars, Primitivo showed the highest symptom severity and bacterial load (TaqMan qPCR). Among table grapes, Baresana and Italia exhibited more severe symptoms, whereas Victoria and Baresana showed higher bacterial concentrations. No linear correlation emerged between bacterial load and symptom severity, suggesting that disease expression is influenced not only by xylem colonization but also by host physiological and defense responses. In our assays table grape variety Crimson seedless showed the lowest symptom severity, while the bacterial load was similar to those found in the other varieties. The symptom severity and bacterial loads of the same plants inoculated in the previous year will be evaluated in the next year in order to assess the effect of the prolonged infection.

P159. A novel and rapid in vitro method for assessment of *Vitis vinifera* susceptibility to *Xylella fastidiosa* subsp. *fastidiosa*

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(See thematic secssion 8.8a)

P160. Functional screening of bacterial isolates reveals candidates for the biological control of *Xylella fastidiosa* subsp. *fastidiosa* in grapevine

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(See thematic session 2.7a)

P161. Preliminary investigations on varietal selection for resistance to *Xylella fastidiosa* in grapevine

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At the beginning of 2024, an outbreak of *Xylella fastidiosa* subsp. *fastidiosa* (*Xff*), the sadly notorious causal agent of Pierce's Disease (PD) of grapevine in California, was intercepted on *Vitis vinifera* in Apulia (Southern Italy). Given the high susceptibility of *V. vinifera* cultivars and the absence of genetic traits associated with resistance to the bacterium in this species so far identified, genetic improvement programs should be activated at the European level to develop resistant grape germplasm. This research aims to analyze sources of resistance belonging to the Vitis genus in order to plan a crossbreeding program, supplemented by phenotypic selection protocols, for the introgression of resistance genes into *V. vinifera* plants. A systematic review of the literature was carried out to identify American species potentially resistant to the pathogen, since it is well known that the natural selective pressure of the pathogen has led native grapes to develop resistance genetic traits. Sources of resistance were found not only in *V. arizonica*, which still remains the most extensively investigated, but also in V. berlandieri, *V. x champinii* hybrid, *V. aestivalis*, *V. rotundifolia*, *V. vulpina, V. mustangensis*, *V. shuttleworthii*, *V. popenoei* and *V. nesbittiana*. Following their identification, different grape varieties were propagated via cuttings and their rooting capacity was also evaluated to ensure a successful integration into the active breeding collection for future controlled crosses. In 2024, a dedicated breeding program was launched, involving crosses between male plants obtained from *V. arizonica* seeds by open pollination and female-flowering of bunch grape cultivars Lambrusco di Sorbara, Picolit, Moscato rosa and Kharistvala tetri. The first filial generation (F1), which comprises 256 hybrids, was subjected to a detailed leaf morphology study using digital image analysis, thus providing a quantitative basis for early selection. On this trend, future perspectives will include the assessment of productive traits of the obtained populations (e.g. sex of the flower, phenology, berries quality) as well as resistance to *Xff* and other common biotic and abiotic stresses that occur in the vineyards, and the employment of additional *Vitis* sources, in order to broaden the genetic base useful for the selection of resistant materials.

P162. The NOVIXGEN project: multidisciplinary strategies for selecting genetic material resilient to *Xylella fastidiosa* and for studying host–pathogen interactions in olive and grapevine

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The NOVIXGEN project aims to limit the spread of *Xylella fastidiosa* by coordinating national Research Units to study host plant tolerance and resistance mechanisms. A multidisciplinary approach combines field monitoring, genetic characterization, and molecular analyses. GIS-based mapping of asymptomatic genotypes in infected areas is followed by morphological, genetic, and phytosanitary characterizations. In three infected sub-zones (Leuca, Gallipoli, Otranto), 200 potentially tolerant/resistant olive genotypes were identified, including 70 asymptomatic spontaneous crosses and 'Cellina di Nardò' specimens with less symptoms. The study also includes a segregating cv. Leccino population (84 genotypes) and 27 'Leccino' clones. Studying the soil microbiome and endophytes aims to identify microbial communities involved in plant defense. Multi-omics platforms investigate host-pathogen interactions, focusing on lipid metabolism, oxylipin genes, and transcriptome modifications in olive cultivars with varying susceptibility ('Leccino', 'Cellina di Nardò', 'Ogliarola salentina'). Parallel anatomy and metabolomics studies analyze xylem tissue changes, lignification, and secondary metabolite accumulation to correlate with tolerance levels. The project develops advanced genomic tools (resequencing, spatial transcriptomics, RNA-targeted sequencing) to identify candidate resistance genes. Investigations extended to grapevine include microbiome studies and artificial inoculations to select *Xylella*-tolerant/resistant Italian varieties; two showed less infection responses. An in vivo microbial consortium was selected that significantly reduces symptoms, highlighting biocontrol and SynComs potential. Furthermore, the first genotypes carrying the PdR1 resistance gene were obtained. NOVIXGEN will continue to work on developing genetic breeding programs for the olive tree and grapevine, which will involve AETs to protect native varieties. All selected material will be planted at the experimental CREA company “Podere Sant'Anna” in Monteroni di Lecce (infected area) to establish a strategic genetic basis for the subsequent phases of the project. This national facility will serve as CREA's center of excellence for *Xylella* research, accessible to the entire national and international scientific community.

P163. *Xylella fastidiosa*: how is Switzerland preparing?

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Switzerland is currently considered free from *Xylella fastidiosa*, but outbreaks in neighbouring countries and the intensification of international plant trade are cause of concern, despite the measures in place for controlling imported plants and the regular surveillance of the Swiss territory. With a proactive approach to the likely arrival of the plant pathogen in Switzerland, Agroscope's Neobiota Competence Centre, in collaboration with the Swiss Federal Institute for Forest, Snow and Landscape Research WSL, are currently developing research for elucidating the epidemiology of *Xylella fastidiosa* in the Swiss context, supporting surveillance and early detection, and suggesting sustainable management strategies. In particular, research is underway that assess the competence and efficiency of insect vectors widespread in the country in acquiring and transmitting *Xylella fastidiosa*, as well as studying the symptomatology of *Xylella* infection on woody plants of agricultural and sylvicultural interest. Past projects have focused on vector *Philaenus spumarius* and have studied its phenology under the insubric climate, identified host plants and their specificity at local and regional scale, and estimated its density in different agroecosystems. Nymphs select preferentially host plants from Asteraceae, Plantaginaceae, Caryophyllaceae and Fabaceae families, in particular Taraxacum officinale, Plantago lanceolata, Silene vulgaris, Trifolium pratense. Nymphs' host preference varies according to regional pools and habitat type. Density of nymphs (spittle nests) is significantly higher in extensively managed habitats and in hay meadows, when compared to vineyards, olive groves, orchards and pastures. Experiments conducted to test management strategies for *Philaenus spumarius* have shown that mowing (and immediate removal of hay) is efficient in reducing vector's abundance, independently of the starting date (period spanning from appearance of first nymph and nymphs' peak). Push and pull approaches influence the spatial distribution of nymphs, the pull bands (attractive host species) showing a higher density of nymphs than push bands (repellent host species), although lower compared to untreated (semi-natural) meadow.

P164. Preparing for *Xylella fastidiosa* in Australia: bridging research, biosecurity and industry readiness

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Xylella fastidiosa poses a substantial biosecurity risk to Australia, threatening high-value industries including viticulture, horticulture, almonds, olives and native plant systems. Although the pathogen is not present in Australia, increasing global trade, plant movement and climate variability elevate the likelihood of introduction and establishment. In response, we have been leading the development of a coordinated national preparedness framework that integrates research, surveillance, vector ecology, risk assessment and industry engagement. This initiative builds on Australia's strong biosecurity system while identifying critical knowledge gaps relevant to local agroecological conditions. Key priorities include understanding potential vector competence of endemic xylem-feeding insects, evaluating climatic suitability and host vulnerability, strengthening diagnostic capacity, and developing surveillance strategies that are both practical and industry-informed. Recent workshops and consultations with federal agencies, state departments, industry bodies and researchers have helped define research priorities and align preparedness efforts with policy and response planning. Given Europe's extensive experience managing *Xylella* outbreaks, collaboration and knowledge exchange are essential. Lessons from European epidemiology, containment strategies, landscape-scale management and stakeholder communication provide valuable guidance for refining Australia's preparedness approach. By sharing our framework and engaging with European colleagues, we aim to strengthen international collaboration and ensure that preparedness strategies are science-based, adaptive, and aligned with emerging global risks.

P165. Mediterranean Cooperation to Contain *Xylella fastidiosa*: RIGENESI

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The epidemic caused by the bacterium *Xylella fastidiosa* in Apulia is one of the most significant phytosanitary emergencies in the Mediterranean over the past century, resulting in profound economic, environmental, and socio-cultural impacts, especially on the olive oil industry and Apulia's historic olive-growing landscape. This crisis underscores the necessity for a coordinated, supranational approach to managing phytosanitary emergencies, considering the cross-border nature of quarantine pests and the climatic similarities across the Mediterranean region. In response, the RIGENESI project reinforces its role in international cooperation for the management of plant health emergencies. Funded by Regione Puglia, it aims to enhance the technical and institutional capacities of Mediterranean countries. The project unites stakeholders involved in the phytosanitary system and applied research through field and laboratory training, institutional dialogues, high-level international events, and policy briefings, focusing on phytosanitary surveillance, laboratory diagnostics, and emergency containment planning. Additionally, RIGENESI fosters structured cooperation among national phytosanitary services and convenes conferences and ministerial meetings to facilitate dialogue between policymakers, the scientific community, and European institutions. These activities support alignment with international phytosanitary standards and the development of a stable Euro-Mediterranean coordination network. The effective management of complex phytosanitary crises requires not only regulatory and scientific tools but also structured mechanisms for knowledge transfer and multi-level governance that are adaptable to different national contexts and that involve stakeholders (e.g., trade associations, farmers, and citizens). This approach advances the alignment of phytosanitary standards and provides a practical model for Mediterranean countries to reinforce preparedness, enhance coordination among phytosanitary authorities, and strengthen regional phytosanitary resilience to transboundary plant health emergencies.

P166. Informative and Awareness-Raising Resources on *Xylella fastidiosa* for Botanic Gardens

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Within the framework of the BeXyl project, botanic gardens have played a key role in plant sampling as sentinel sites for early detection of plant pathogens. Their contribution, however, extends beyond surveillance: botanic gardens are uniquely positioned to support public engagement, in education and awareness-raising regarding plant health, biosecurity practices, and the early detection of emerging threats such as *Xylella fastidiosa*. To support this role, a comprehensive resource kit was designed and developed under the BeXyl project for botanic gardens and their educators. The kit aims to facilitate outreach to diverse audiences, including garden staff and volunteers, school and university visitors, and other communities of interest, such as agriculture. It includes a variety of materials, including informational posters highlighting the role of botanic gardens as early warning systems, practical guidance on biosecurity procedures, and accessible content introducing *Xylella fastidiosa* and its impacts. In addition, the kit provides audience-tailored tools to promote understanding and engagement with plant health and biosecurity, interactive activities to illustrate the impacts of the pathogen, adaptable presentations for educational use, and social media templates to extend and disseminate key messages more widely. The key objective of these resources is to raise awareness and deepen understanding of *Xylella fastidiosa* and its impacts, ensuring that the risks associated with the bacterium and the role of botanic gardens are more widely understood. At the same time, the resources highlight and promote the adoption and communication of best biosecurity practices. Ultimately, contributing to the protection of plant health, conservation of biodiversity and support of resilient ecosystems.

P167. Beyond *Xylella*, Integrated Management Strategies for Mitigating *Xylella fastidiosa* impact in Europe (BeXyl)

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Xylella fastidiosa represents one of the most serious biological threats to Mediterranean agriculture and landscapes, severely affecting economically and ecologically important crops such as olive, grapevines and almonds. The Horizon Europe-funded BeXyl project constitutes a coordinated European response to this challenge, bringing together a multidisciplinary and multi-actor consortium to develop integrated solutions for the prevention, early detection and sustainable management of *X. fastidiosa* outbreaks across the EU. BeXyl addresses the pathogen through a comprehensive framework that combines epidemiology, diagnostics, surveillance, host resistance, biological control, integrated pest management (IPM), socioeconomic analysis and policy support. BeXyl's strategy is structured around three complementary pillars: (i) strengthening preparedness through improved prevention, surveillance and rapid response capacities; (ii) developing sustainable management strategies based on resistant or tolerant plant material, biological control agents, vector management and IPM approaches; and (iii) generating scientific evidence to support risk assessment, plant health regulations and decision-making processes within the EU and associated countries. Key project activities include the development and validation of advanced molecular diagnostic tools, the implementation of remote sensing technologies for large-scale surveillance, the establishment of sentinel plant networks for early detection, and the creation of innovative phenotyping platforms to assess host responses under field and controlled conditions. BeXyl has also advanced the identification and characterization of resistant and tolerant germplasm in major host crops, particularly olive, almond and grapevine, providing new opportunities for long-term disease management and adaptation. In parallel, the project is evaluating novel biologically based solutions, including synthetic microbial communities, bacteriophages, antimicrobials and plant-derived compounds and entomopathogenic microorganisms targeting insect vectors. Beyond technical and biological innovations, BeXyl investigates the socioeconomic, environmental and governance dimensions of X. fastidiosa outbreaks, assessing the impacts of different management strategies, stakeholder perceptions, barriers to implementation and the acceptability of control measures. These analyses contribute to the development of evidence-based and socially acceptable IPM strategies tailored to different outbreak scenarios and production systems. Through the integration of research, innovation, stakeholder engagement and policy support, BeXyl contributes to the objectives of the EU Plant Health Law and the European Green Deal, enhancing Europe's capacity to prevent new outbreaks, mitigate the impact of existing epidemics and strengthen the long-term sustainability, resilience and competitiveness of European agriculture and forestry.

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