BIOVEXO

Biocontrol of *Xylella* and its vector in olive trees for integrated pest management

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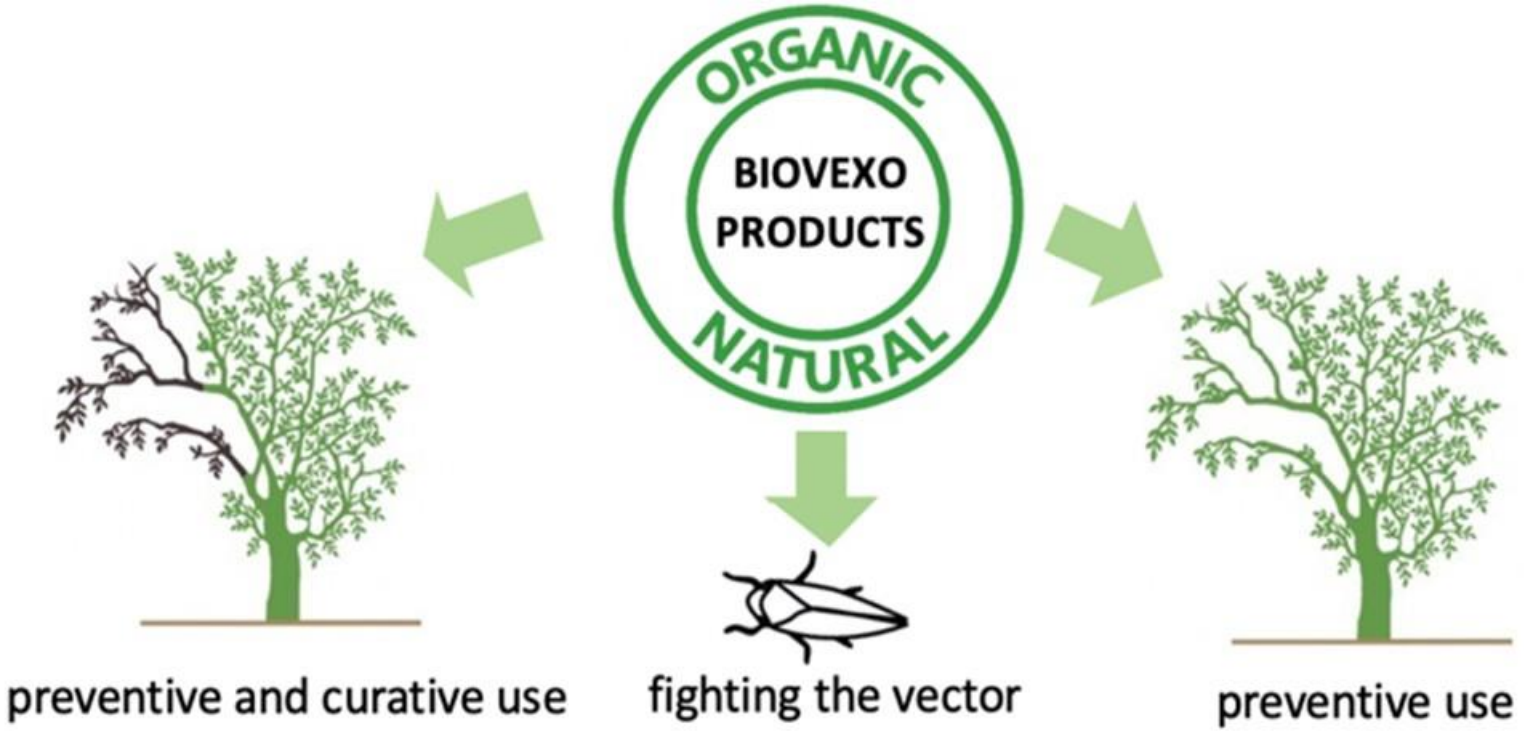
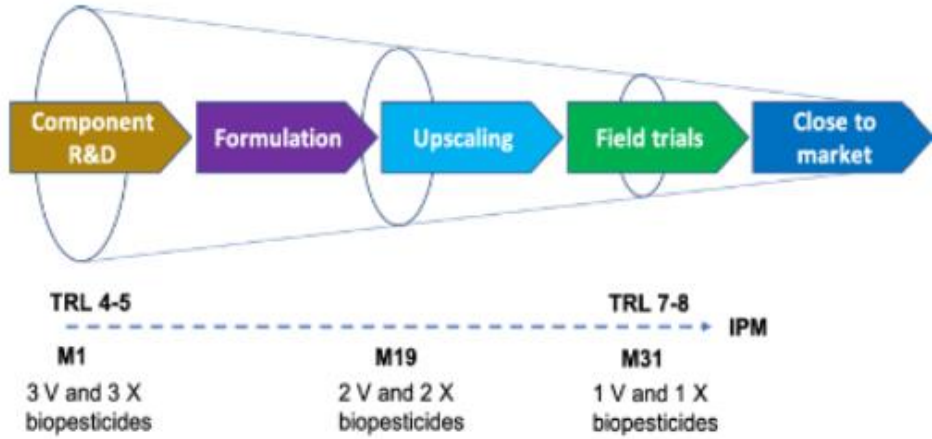
This project has received funding from the Bio Based Industries Joint Undertaking (JU) under grant agreement No 887281. The JU receives support from the European Union's Horizon 2020 research and innovation programme and the Bio Based Industries Consortium.

BIOVEXO demonstrates environmentally sustainable and economically viable plant protection solutions, combining the use of *Xylella*-targeting biopesticides (X-biopesticides) with biopesticides combatting the insect vectors transmitting the disease (V-biopesticides), and makes them available for ready use in integrated pest management.

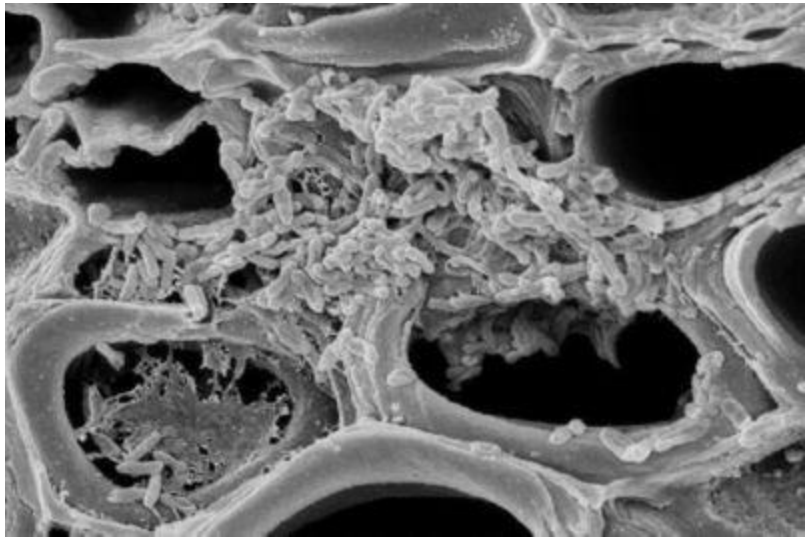




Xylella fastidiosa

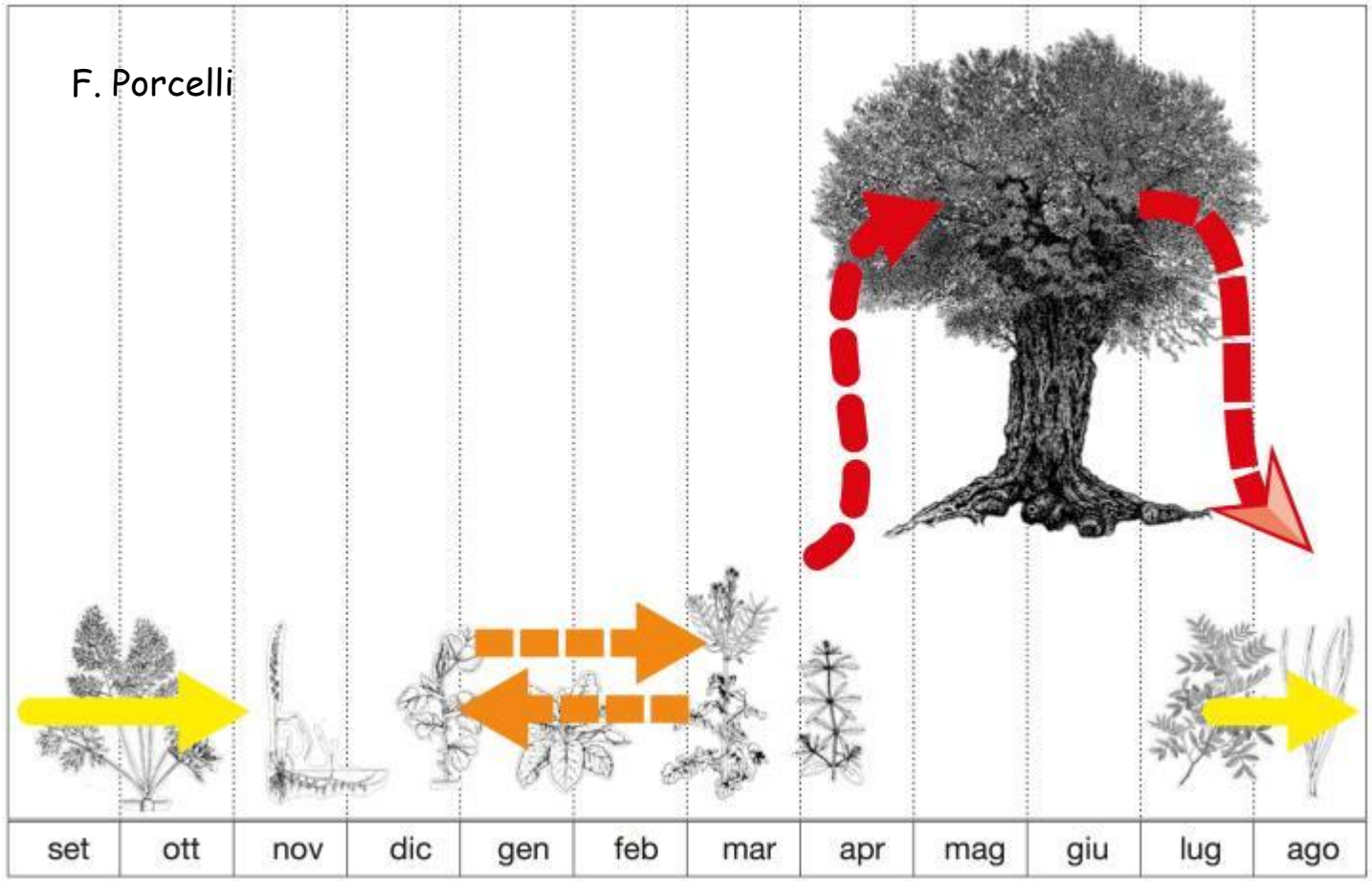
- Vector-borne bacterium
- Forms biofilms in the xylem of the host plant
- Transmitted by xylem-feeding insects, mainly spittlebug *Philaenus spumarius*
- Causes diseases in many plants of economic interest

TEM micrograph of *Xylella fastidiosa* in the xylem vessel of olive and spreading through A pit membrane , Montilon et al. 2022



Scanning electron micrograph of *Xylella fastidiosa* in the xylem vessel of a orange, E. W. Kitajima

Biological cycle of *P. spumarius*



Olive is the main host of *Ph. spumarius* and the major inoculum source for the transmission of *Xylella* from plant to plant

Xylella epidemic on olive in Apulia

Olive quick decline syndrome

Symptoms range from leaves desiccation to death of the plant in susceptible cultivars

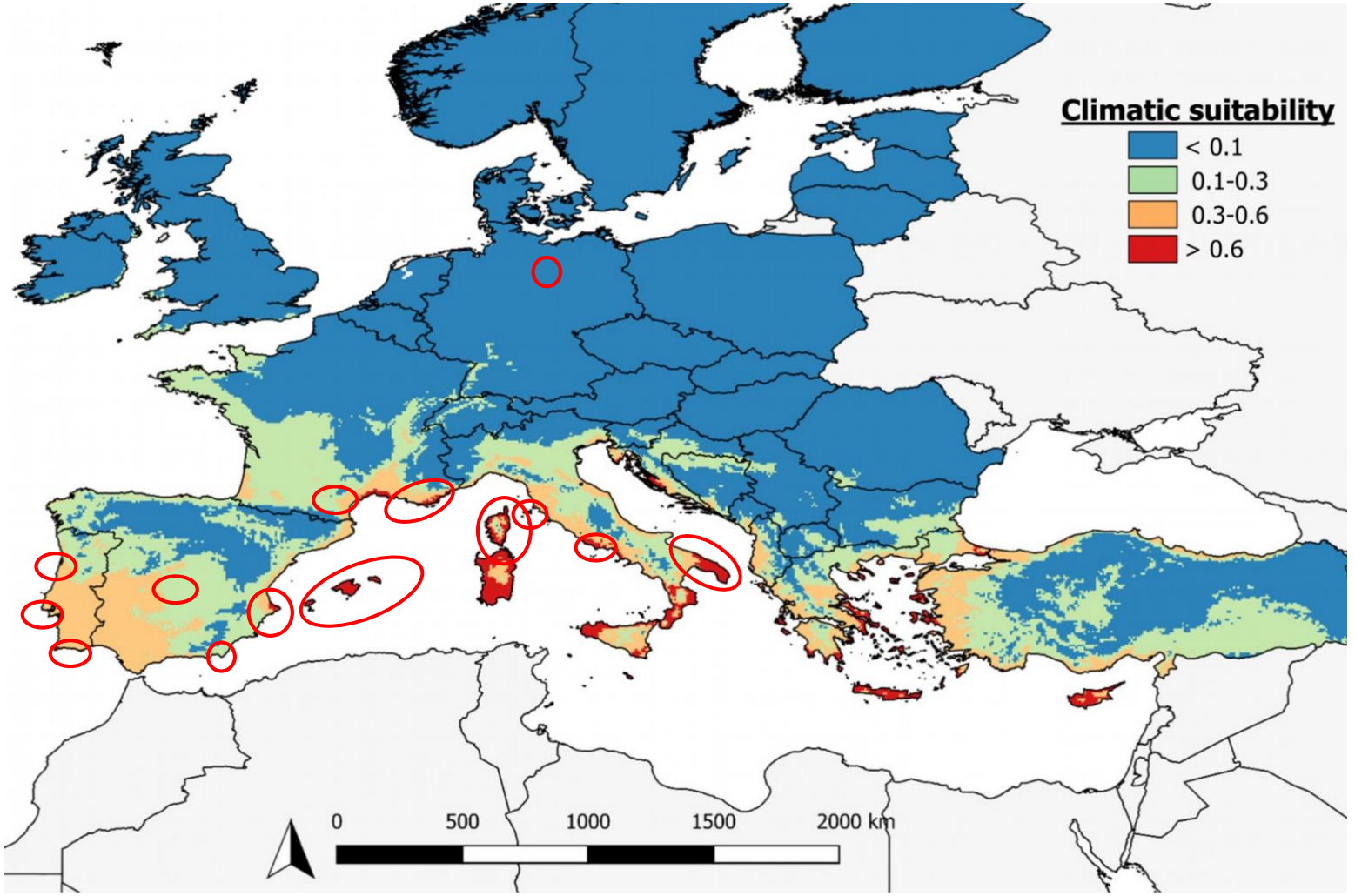
Estimated yield losses

Crop	Estimated yield loss (median)
Olive trees younger than 30 years	34.6%
Olive trees older than 30 years	69.1%
Almond	13.3%
Wine grape in southern EU	2.1%
Table grape in southern EU	1.0%
Wine grape in northern EU	0.5%
Citrus spp.	10.9%

Estimated yield losses should Xylella become widespread in Europe. From: EFSA Journal 2019;17(5):5665

Xylella fastidiosa = quarantine/priority pest

2013	Puglia, **Italy**
2014	Iran
2015-16	Corsica/Mainland, **France**
2016	**Germany**
2016-17	Balearic Island/Alicante, **Spain**
2017	Israel
2018	Madrid & Almería, **Spain**
2018	Tuscany, **Italy**
2019-21	**Portugal**
2020	Occitania, **France**
2021	Lazio, **Italy**
2022	

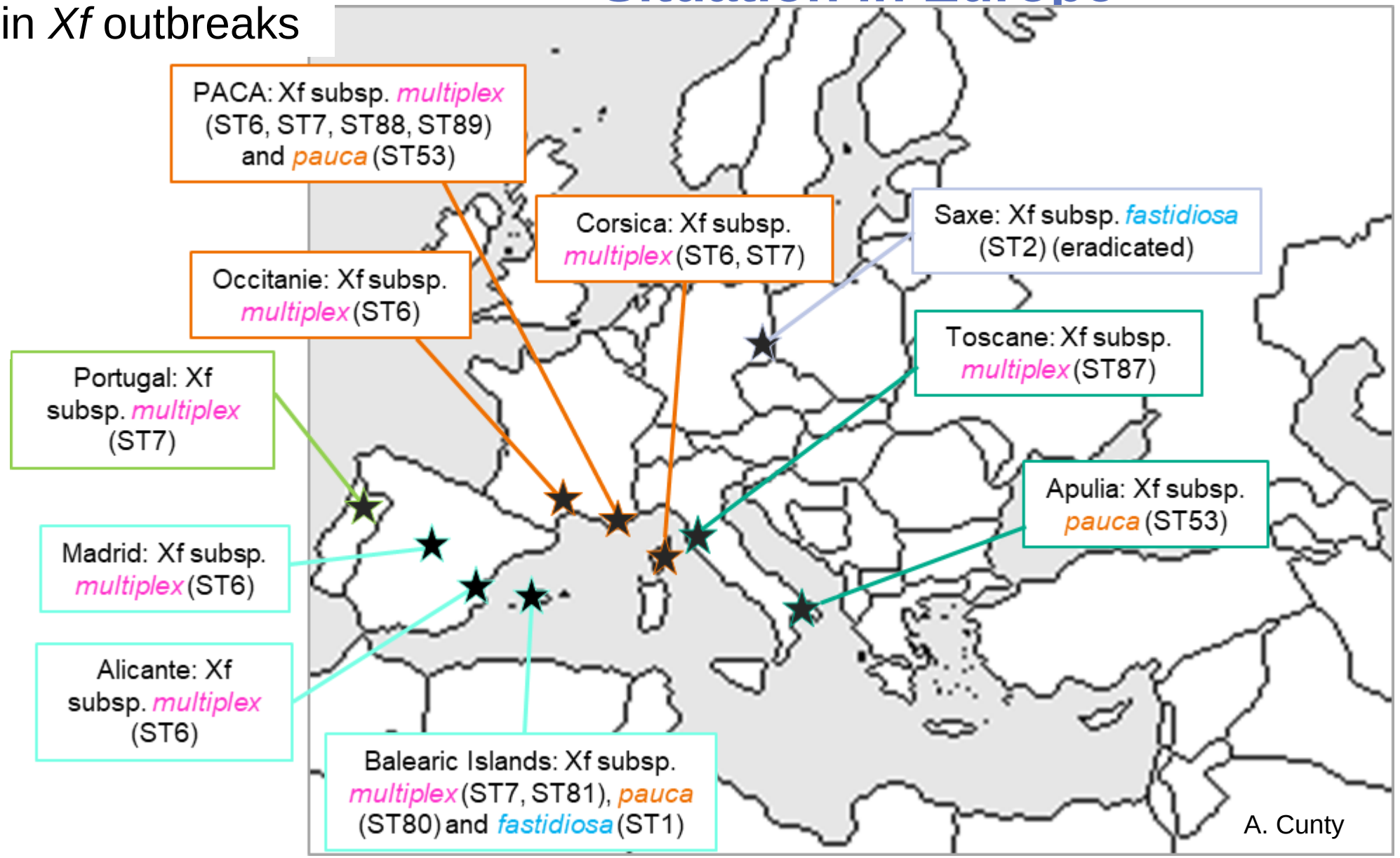


Source: EFSA PLH Panel, 2019. EFSA Journal 17(5):5665

Situation in Europe

High genetic diversity of the strains spreading in *Xf* outbreaks

Preserve further spread, cross-contamination



Xylella epidemic on olive in Apulia

Different stages of the Olive Quick Decline Syndrome

The «Gigante di Alliste», a 1500 years old monumental tree in Alliste, Salento

BIOVEXO
Project overview

As a response to the increasing *Xylella* outbreaks in Europe, the BIOVEXO Project explores innovative biopesticides, which target the *Xylella* bacterium.

Six candidate biocontrol solutions acting either against *Xylella* or its vector will be tested within the BIOVEXO Project:

- two bacterial strains
- a microbial metabolite
- two plant extracts
- an entomopathogenic fungus

Horizon 2020 European Union Funding for Research & Innovation
BIO-BASED INDUSTRIES Public-Private Partnership
Bio-based Industries Consortium
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BIOVEXO

Partners

RTDS ASSOCIATION
www.rtds-group.com

CRSFA Centro di Ricerca
www.crsfa.it

AIT Austrian Institute of Technology
www.ait.ac.at

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Consiglio Nazionale delle Ricerche
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DOMCA
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AciesBio
www.aciesbio.com

AIMERIT SL

Asociación Agraria Jóvenes Agricultores
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Acknowledgement

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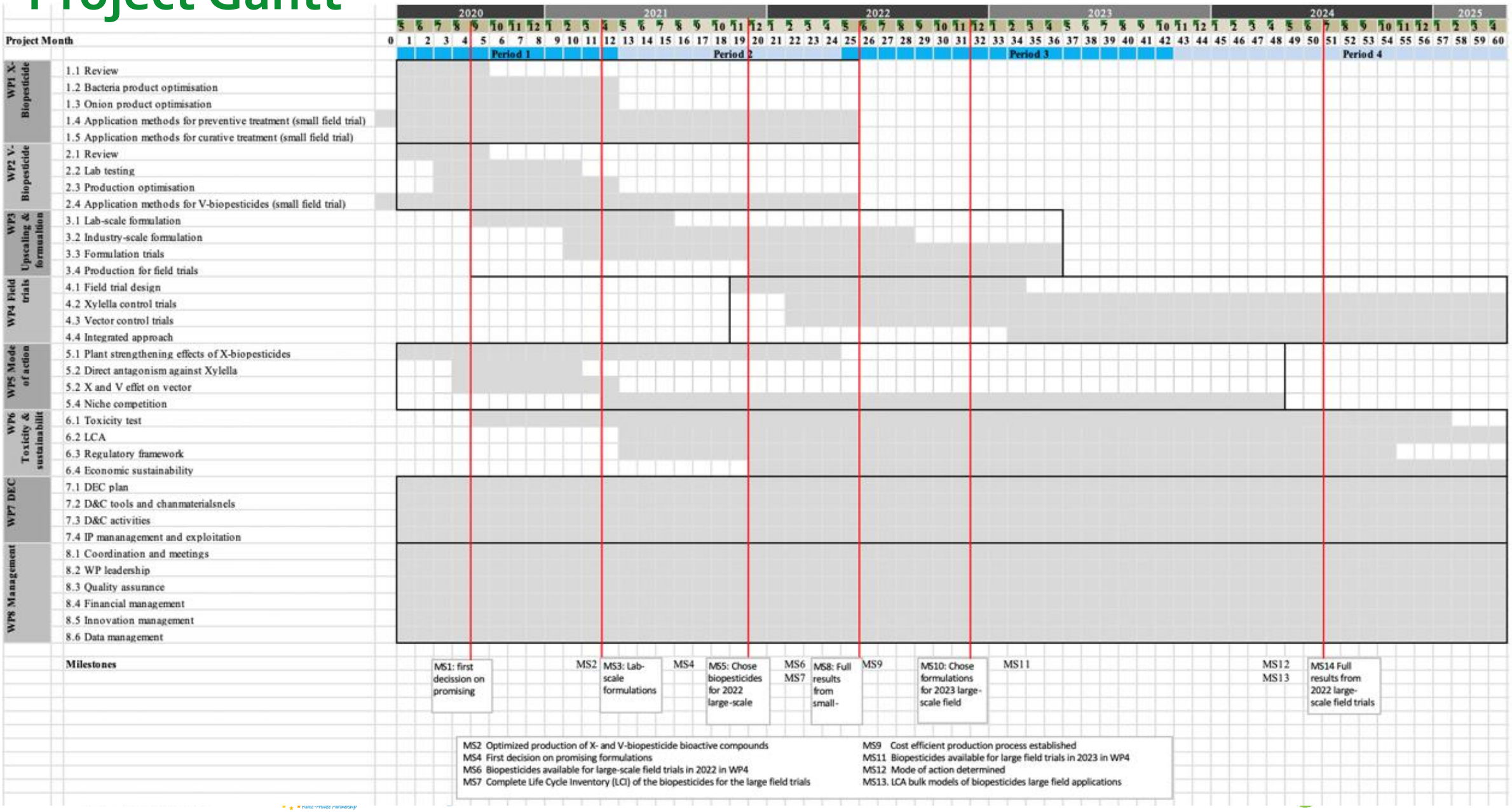
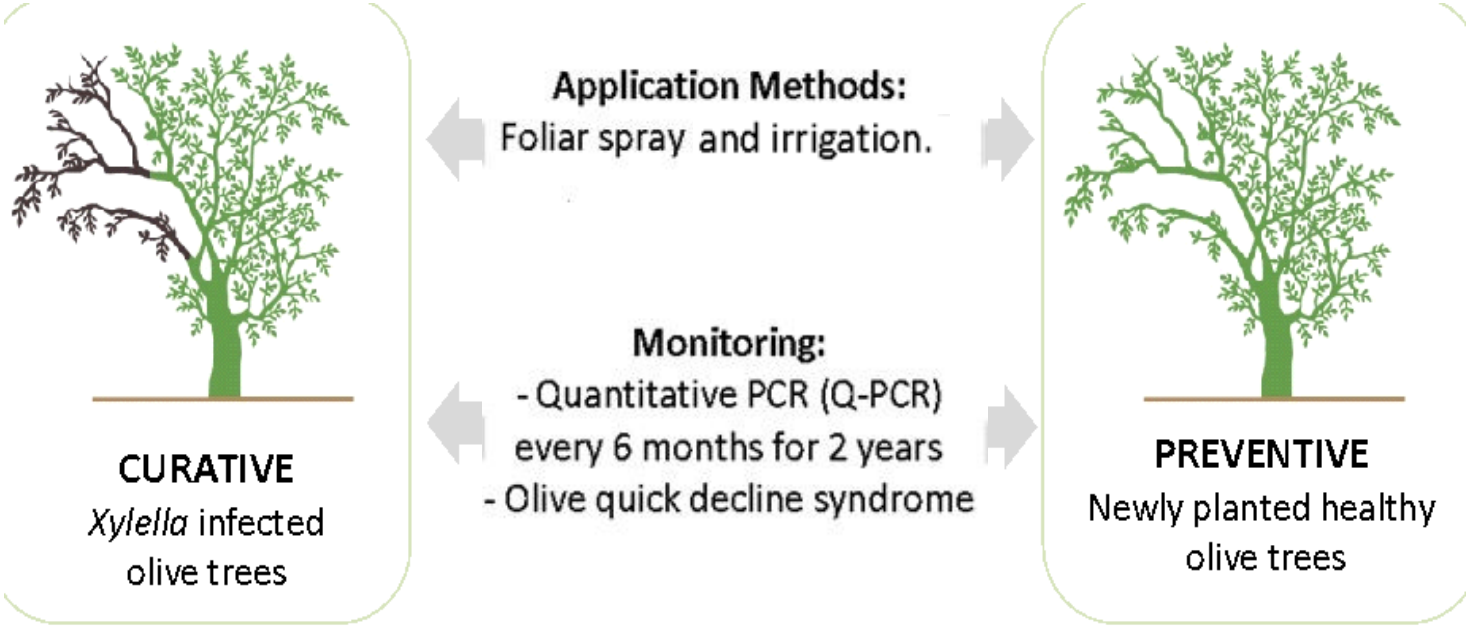
Project Gantt



X-biopesticides for Xylella control

- Beneficial bacterium colonizing plants endophytically with proven activities against *Xylella* in grapevine
- Beneficial bacterium having antagonistic activities against various pathogens affecting olive trees
- Plant extract reducing *Xylella* infection *in vitro* and soil borne fungi infections in olive trees



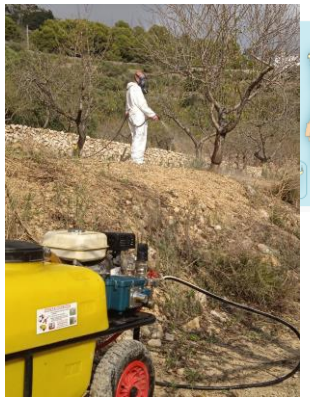
WP1

Work Package led by:

X-biopesticides for Xylella control

DOMCA
INNOVATIVE FOOD SOLUTIONS

- Selection of biosolutions for *Xylella* targeting X-biopesticides
- Optimization of production
- Testing of biosolution in 2 locations:



Olive orchards in Apulia

Almond and olive orchards in Mallorca and Alicante

curative

preventive

- Identification of best application methods of biocontrol solutions for Xylella prevention and curative approaches

c. 200 plants /trial
Brindisi, 2 orchards
Latiano, 1 orchard
Avetrana 2 orchard
randomized blocks

WP2

V-biopesticides for vector control

- Production of active substances for vector targeting V-biopesticides
- Identification of best application methods for preventing *Xylella* infections
- Lab testing of V-biopesticides against the different life stages of insects
- Establishment of a protocol for optimized production
- Small-scale field trials in Apulia, Italy

Work Package led by:

Globachem
Your Crop Counts

Agrochemical company specialized in developing, registering and marketing high quality crop protection products for agriculture and horticulture.

V-biopesticides for vector control

- Entomopathogenic fungus killing spittlebugs
- A microbial metabolite active against spittlebugs
- A plant extract active against spittlebugs

Weeds growing adjacent to olive plantations
targeting the juvenile vector

Olive trees
targeting the adult vector

- Insect mortality and population sizes were monitored in dependence of application mode
- Time and dose concentration were evaluated

WP3

Work Package led by: DOMCA INNOVATIVE FOOD SOLUTIONS

Biopesticide formulation and upscaling

- Formulation of X- and V- biopesticides from lab-scale to industry scale, which includes production for field trials.
- Development of parameters of bio-process control
- Evaluation of optimal efficacy and economic viability.

1. Optimization of formulations on a laboratory scale
- Adjuvant and protection technologies (emulsions, lyophilization, encapsulation or microencapsulation)
- Prototype formulations

2. Upscaling and optimization of formulation on an industrial scale
- Upscaling of bioprocess and downstream processes
- Stability and shelf life studies
- Prototype biopesticides

3. Small-scale field trials with different formulations
- Field trials to test X and V- biopesticides formulations, studying safety aspects and effectiveness in planta.

4. Biopesticide production for large field trials
- X- biopesticides
- V- biopesticides

Biopesticide formulation and upscaling

- Formulation of X- biopesticides for endotherapy application.
- Evaluation of appropriate components.
- Phytotoxicity tests carried out in experimental greenhouse conditions

WP4

Work Package led by:

Consiglio Nazionale delle Ricerche

Large scale validation of control strategies for integrated pest management

- Testing of X and V- biopesticides in real field management conditions in Southern Italy and mainland Spain
- Large scale field trials to reduce bacteria spread
- Large scale field trials for Xyella control
- Assessment of integrated control strategies
- Recommendation for integrated pest management strategies

2022-2025

Large scale validation of control strategies for integrated pest management

- 2 preventive trials – foliar and drip irrigation
- 4 curative trials – foliar, drip irrigation and endotherapy
- 2 X-biopesticides, 1 V-biopesticide
- 72 - 432 plants / trial
- Development of a IPM strategy using X- and V-biopesticides

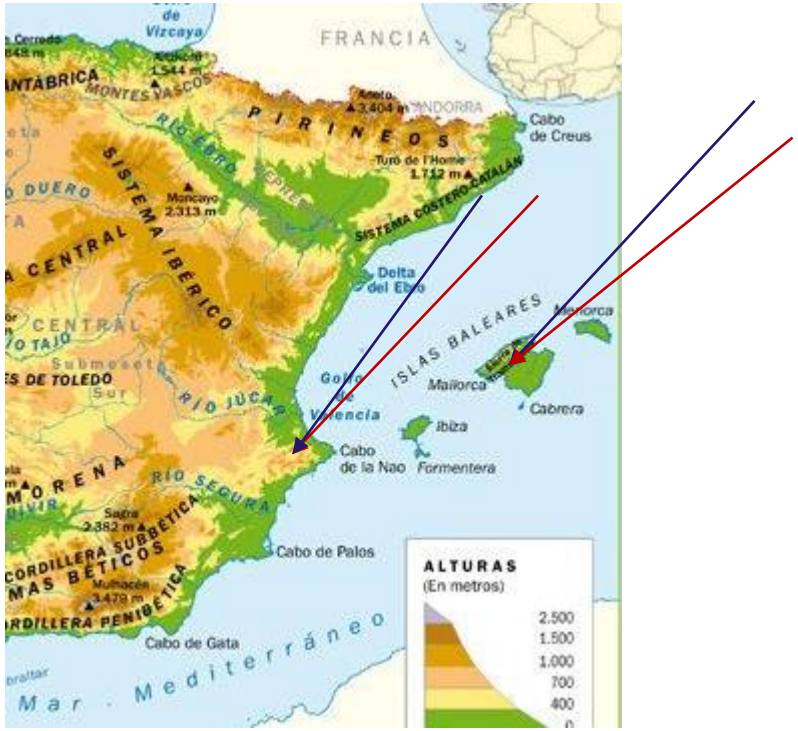
Large scale validation of control strategies for integrated pest management

Olive

- 1 preventive trial – foliar and drip irrigation
- 1 curative trial – foliar and drip irrigation

Almonds

- 2 preventive trial – foliar and drip irrigation
- 2 curative trial – foliar and drip irrigation



WP5

Work Package led by:

AUSTRIAN INSTITUTE OF TECHNOLOGY

Mode of action of biopesticides

- Evaluation of plant strengthening effects
- Definition of mode of action of X and V- biopesticides
- Studying behaviour of applied biopesticides
- Support in product development and registration

X-biopesticide antagonism in vitro against *Xylella*	X-and V-Colonisation of spittlebugs	X- and V-boosting of immune Plant defense reaction	X-Microbial niche colonisation inside plants- *Xylella* competition

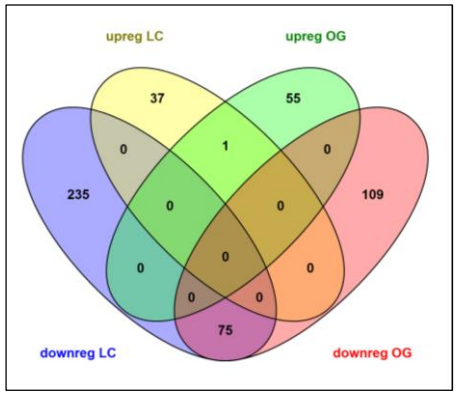
→ Characterisation of direct and indirect MOA for registration dossiers + persistence of biopesticides

Mode of action of biopesticides

Greenhouse trials

In the case of microbial agents:

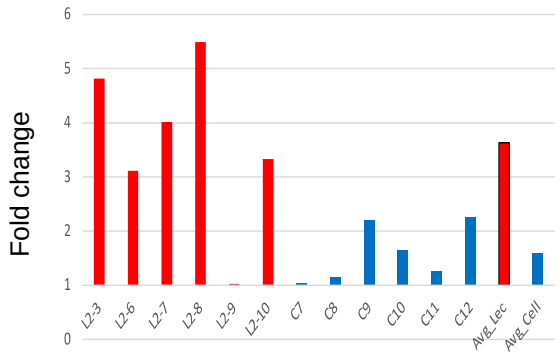
- Quantification of Xylella by qPCR in the plant and in the vector.
- Survival of microbial agent in the vector by qPCR, confocal microscopy and fluorescence in situ hybridisation methods
- Persistence of endophytes inside plants by qPCR.
- Plant defense induction by gene expression analysis.



RNASeq

For extracts and metabolites:

- Determination of minimum bactericidal concentration *in vitro.*
- Quantification of *Xylella* by qPCR in the plant and in the vector.
- Plant defense induction by gene expression analysis.



Expression of selected genes

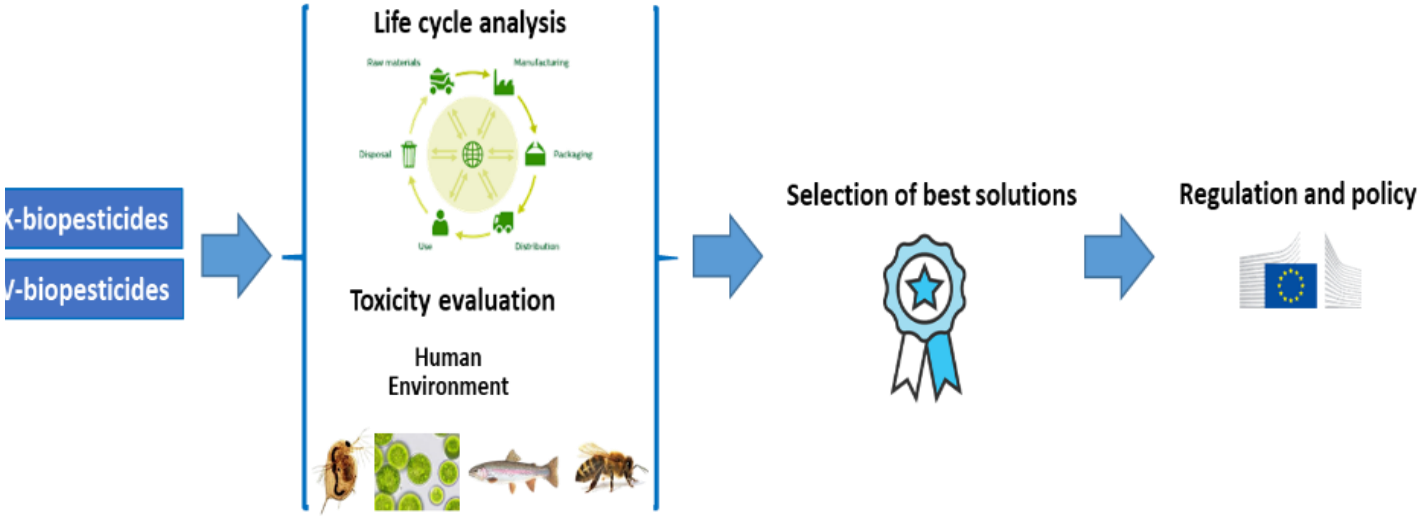
WP6

Work Package led by:

Globachem
Your Crop Counts

Product sustainability and toxicity

- Analysis of the economic sustainability, to ensure regulatory compliance and safety
- Evaluation of the environmental impacts of BIOVEXO biopesticides compared to existing pesticides
- Comparative life cycle assessments (LCA) of conventional pesticides and BIOVEXO biopesticides



WP7 & 8

Dissemination, Exploitation & Communication

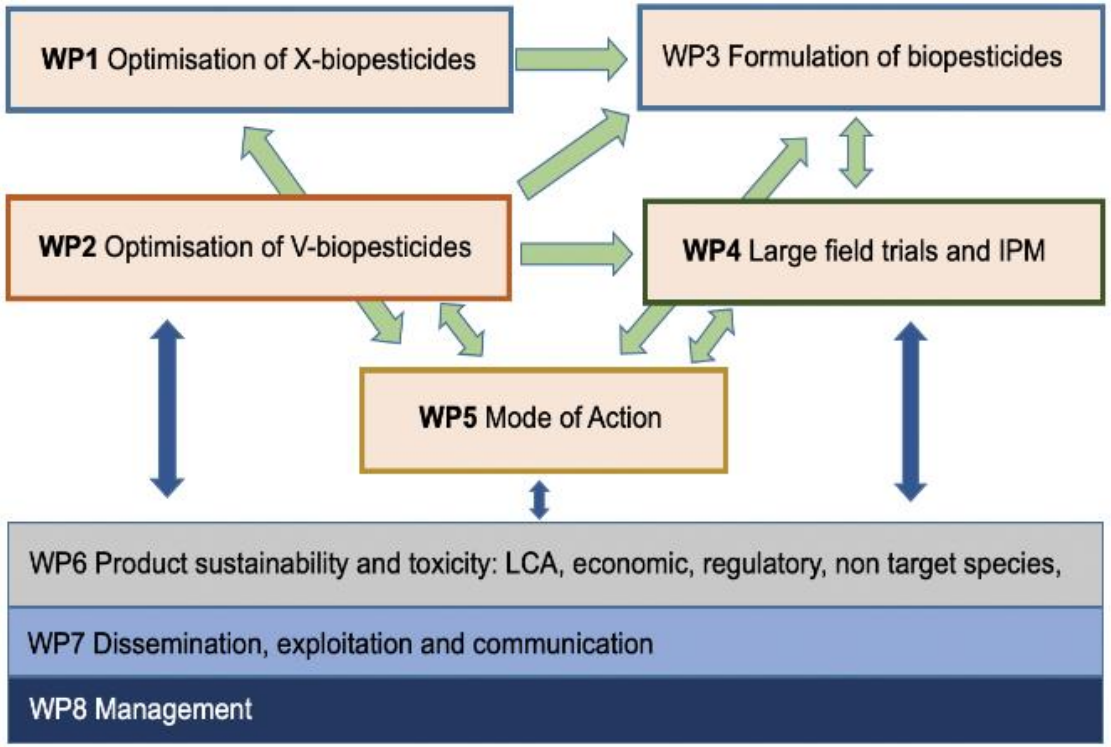
- Development & implementation of a DEC strategy to enhance the innovation capacity of the Biovexo Project
- Continuous communication of BIOVEXO's objectives
- Dissemination of project results
- Establishment and implementation of communication tools (e.g. website, social media channels, social media videos, etc.) and building relevant audiences to maximise impact of the Project
- Establishment of a Project community through DEC activities
- IP Management and Exploitation planning

Management

- Monitoring of the BIOVEXO project & active risk management
- Overall coordination and organization of partner meetings
- Facilitation and implementation of a common strategy
- Management of administrative tasks (internal reporting, periodic cost reporting, deliverables, etc.)
- Communication with the funding authority BBI-JU
- Data management
- Financial management
- Project Coordination: RTDS Association
- Scientific Coordination: AIT Austrian Institute of Technology

Work Package led by:

RTDS GROUP



BIOVEXO

Environmental benefits

- No chemical products
- Protect non-target organisms like bees
- Suitable for organic production
- Reduced exposure to harmful substances

We'd love to hear from you. Here's how you can contact us!

Write us at biovexo@rtds-group.com

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Thanks for your attention

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