



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

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REGIONE PUGLIA

Project Information

BIOVEXO

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[Project website](#)



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Austria



Horizon 2020
European Union Funding
for Research & Innovation



Bio-based Industries
Consortium

11 partners (industries, smes, academia, farmer association) from
Austria, Spain, Italy, Belgium, and Slovenia



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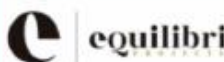
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BIOVEXO

Project objectives

The BIOVEXO Project aims to produce and test innovative biopesticides, bringing them to a TRL 7-8 (close to market entry), specifically:

- Reducing *Xylella* pressure and disease severity in olive cultivation
- Protecting and securing long-established olive orchards
- Preventing *Xylella* infections and disease in new olive plantations
- Securing and sustaining jobs in disease affected areas
- Ensuring continued olive and almond cultivation



BIOVEXO in a nutshell



Jose M. García Madero

X-biopesticides selected
small-scale trials



Alberto Baños Arjona

production optimized at laboratory scale
production upscaled at industrial scale



Dr. Liesbeth Zwarts

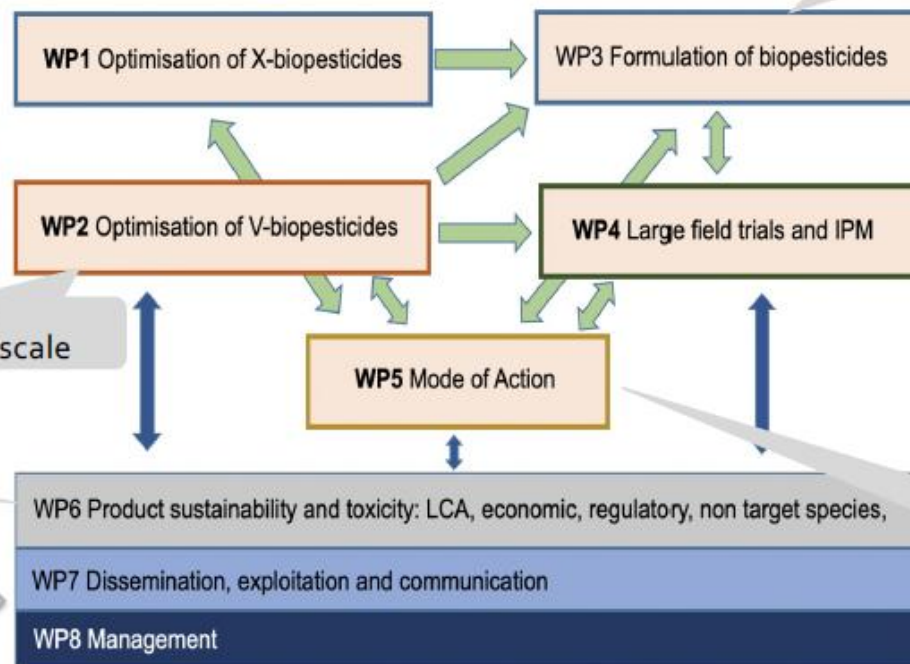


V-biopesticide
selected small-scale

life cycle, toxicity,
regulatory, sustainability



Dr. Stephen Webb



Monitoring efficacy in IPM
strategy



Pasquale Saldarelli

Genomic, RNA seq, advanced
microscopy, colonization in
planta /insects



Stéphane Compant

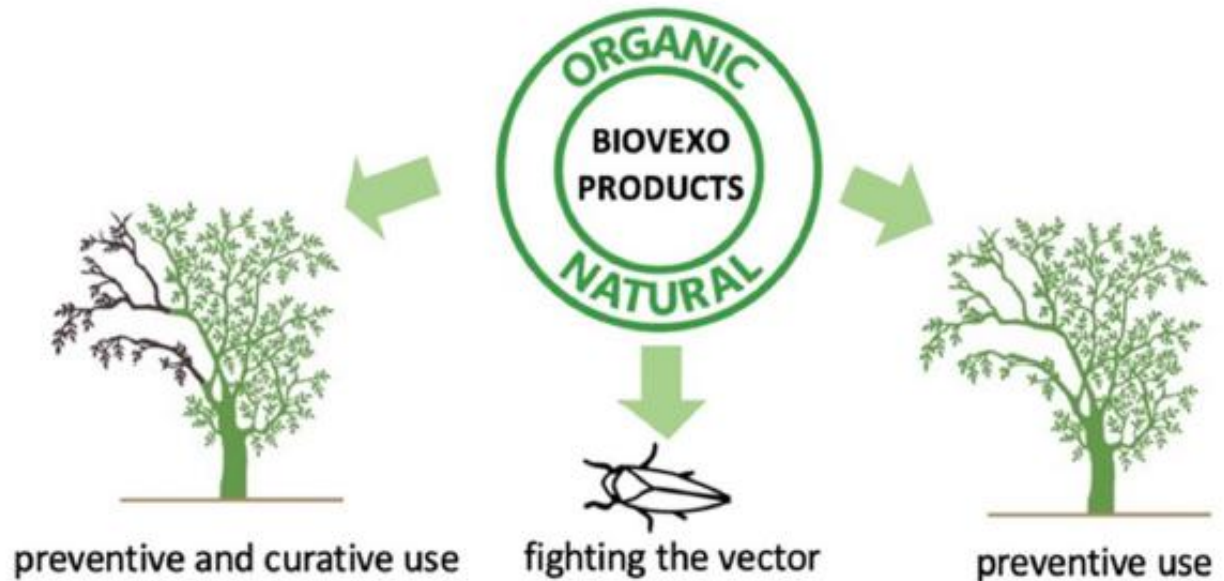
Project Overview

- The BIOVEXO Project explores innovative biopesticides, which target the *Xylella* bacterium.

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

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

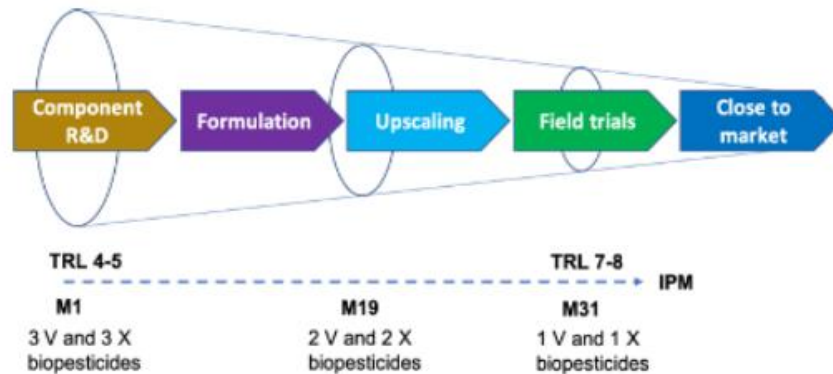
Solutions from our pipelines start at TRL 4-5



Results obtained so far



- Upscale and formulation of biopesticides
- Tests performed on olive orchards and almonds against *Xylella fastidiosa* or its vector.
- Spray, drip irrigation or endotherapy tested
- Toxicology tests done
- Life Cycle Analysis done
- Mode-of-Action determined



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

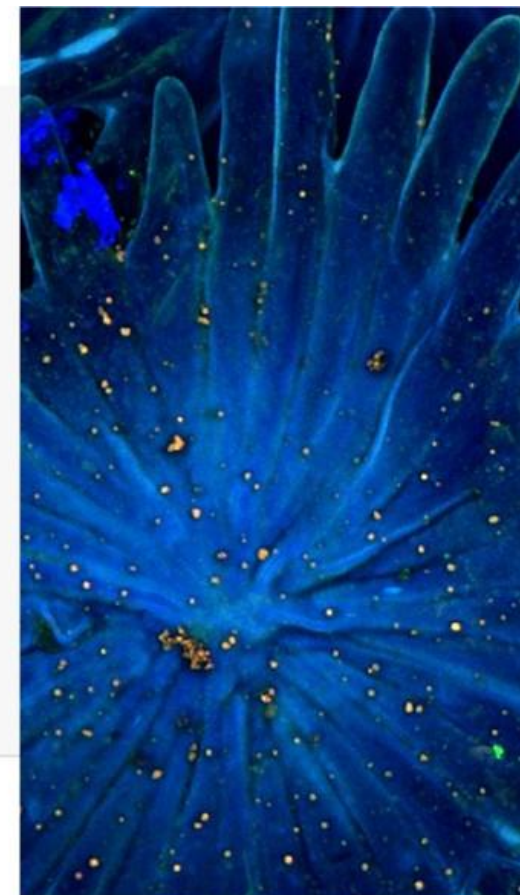


Genomic, RNA seq, advanced microscopy, colonization in planta /insects

Work Package led by:



Stéphane Compant



X-biopesticide
antagonism in vitro
against *Xylella*

X- and V-
Colonisation
of spittlebugs

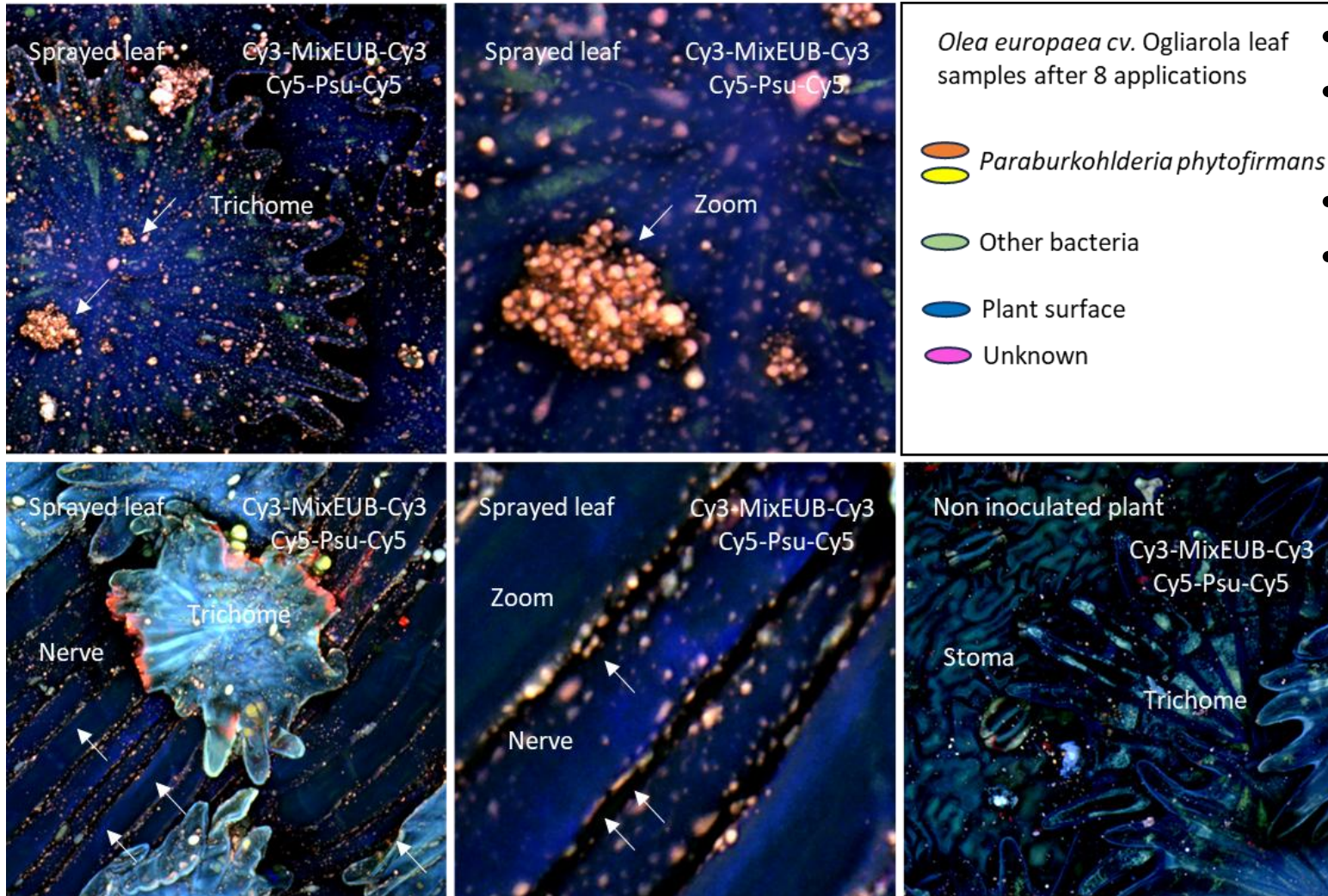
X- and V-boosting
of immune Plant
defense reaction

X-Microbial
niche colonisation inside
plants- *Xylella* competition



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

Plant colonization by PsJN



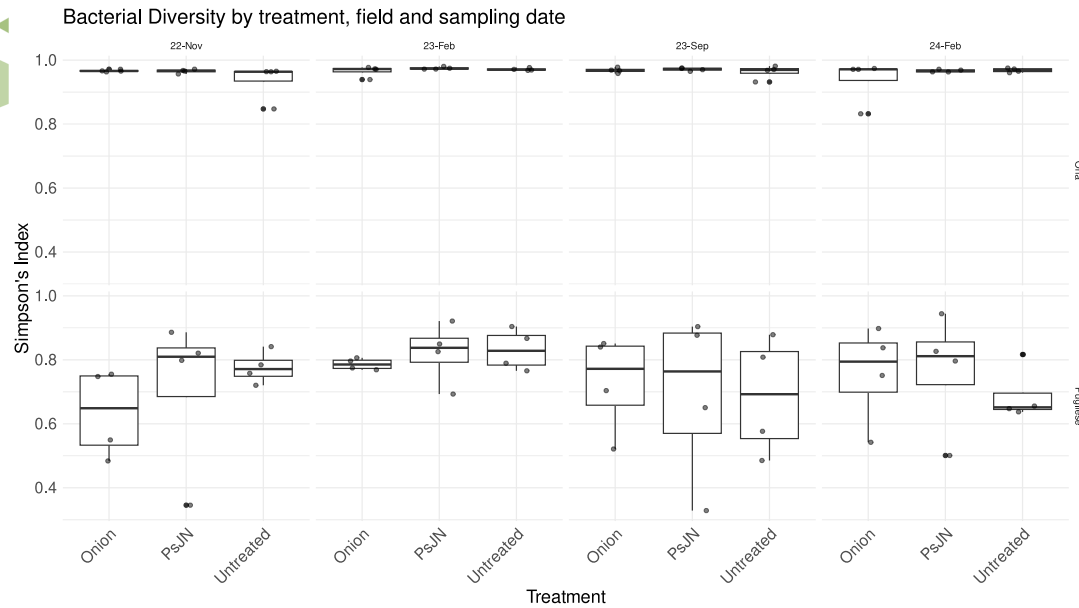
- **No endophytism**
- **Detection only on surfaces of leaves**
- **Low population (qPCR):**
- **log 1-3, 1 month after 8 last applications**

In the field:

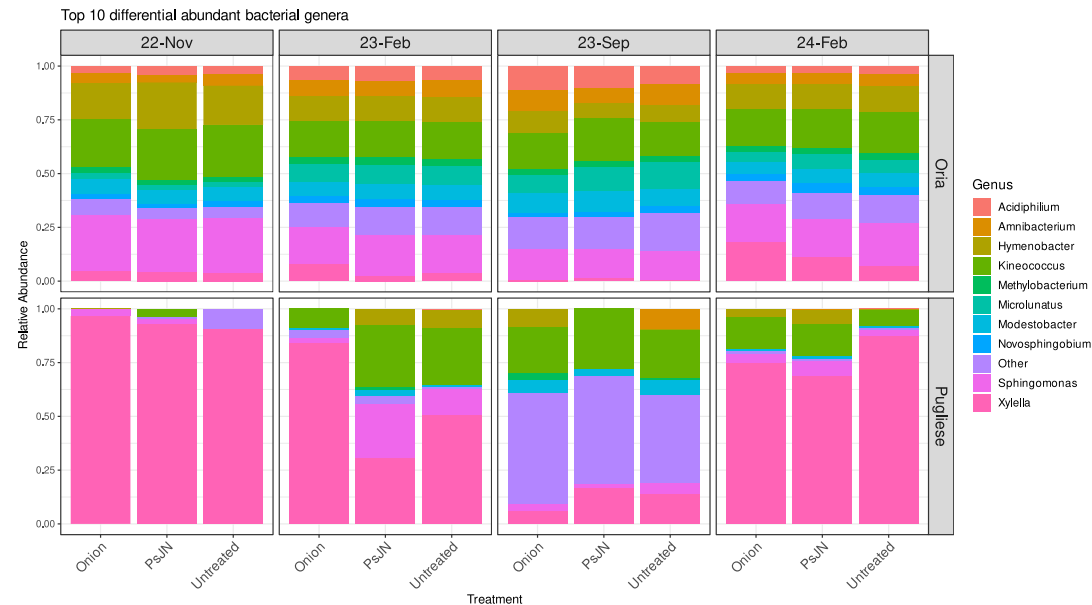
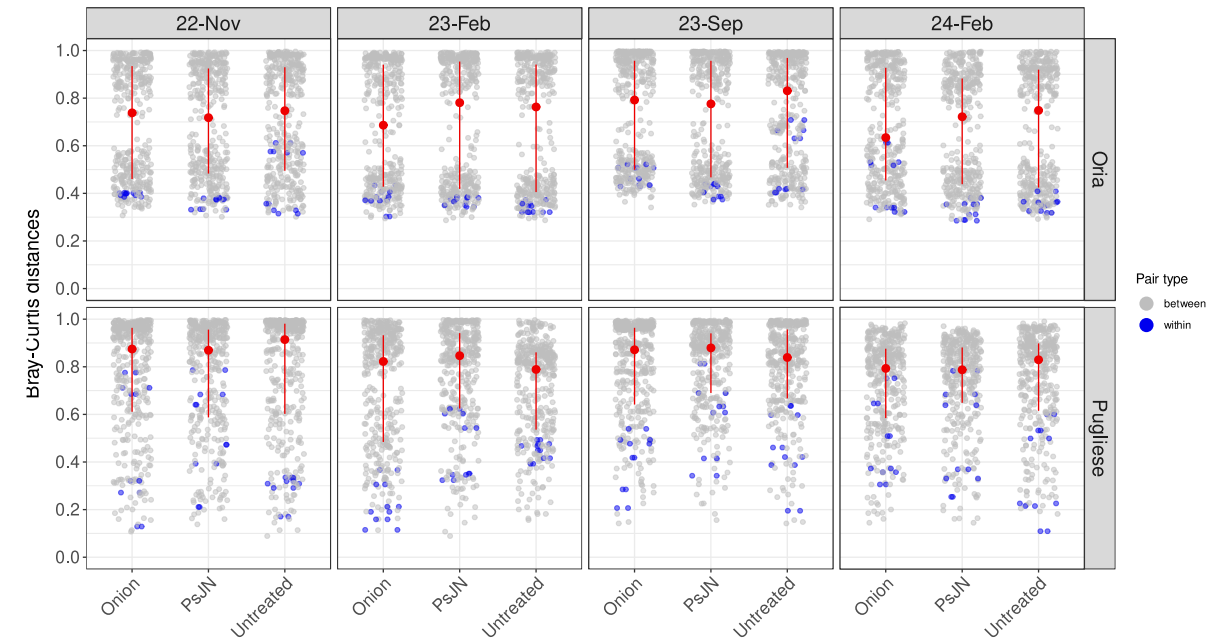
Low population (qPCR) log 1-3 depending of month, year and location

**Sometimes, no detection.
PsJN does not survive
on olive plants.**

Plant microbiome

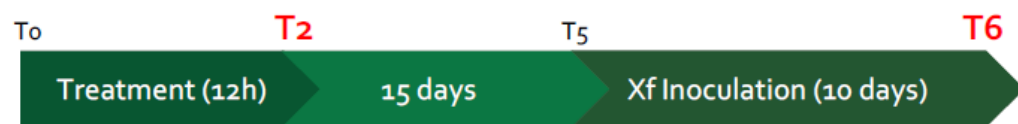


Jitter plot of bacterial Bray-Curtis dissimilarities (within/between groups, 1000 rarefactions)



- Small effect of formulated PsJN on fungal diversity not bacteria
- Fields are the main drivers of alpha-diversity.
- Significant effects on community structure were found for Field, Time, and their interaction, but not for Treatment or its interaction terms.
- Field, Time, and treatment affects some microbial abundances.

Plant transcriptome

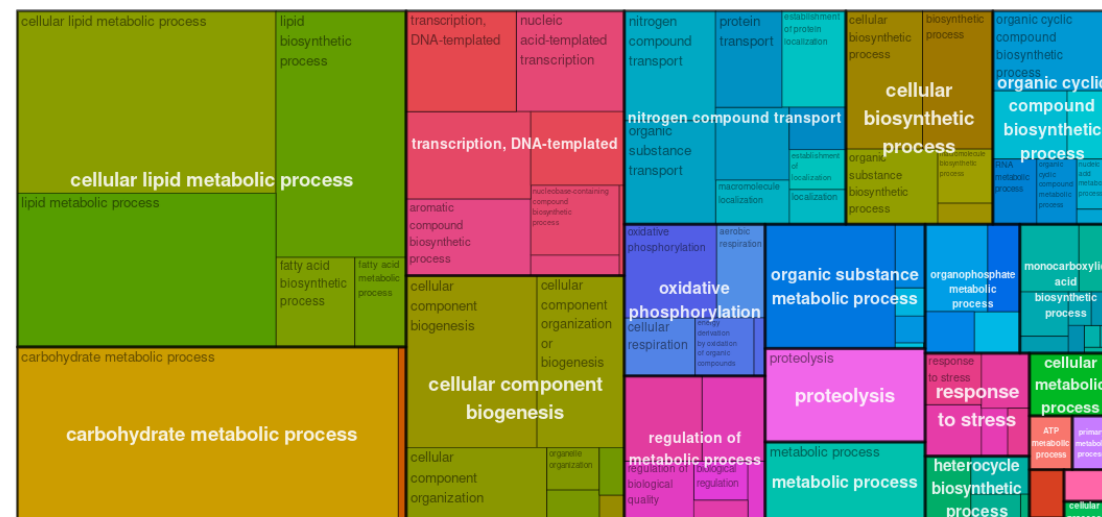


- 36 plants
- 6 treatments (mock, Biopesticide 1, 2, 3)
- 7 time points (To - T6)
- *Xf* infected/uninfected
- RNA extraction
- **RNA sequencing**

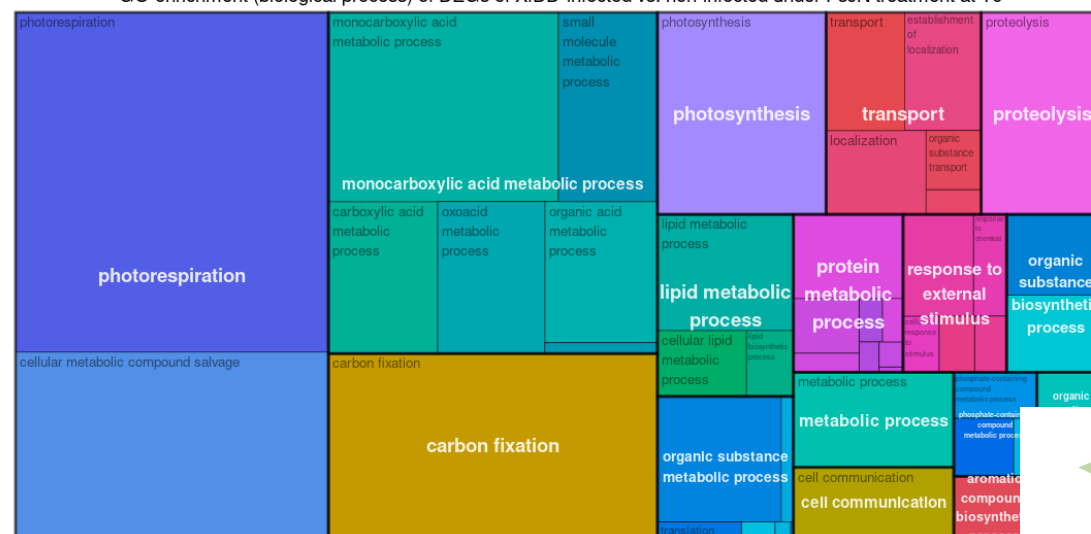
Time point	Treatment / <i>Xf</i>	Gene Ontology
T6 (10dpi)	<i>Xf</i>	response to stress, response to biotic stimulus
T6 (10dpi)	<i>Xf</i> + Biopesticide 1	plant growth stimulation (photorespiration; RuBisCO activity)
T6 (10dpi)	<i>Xf</i> + Biopesticide 3	defense response, transcription
T6 (10dpi)	Biopesticide 1	stimulation of plant immunity, plant growth promoter activity

Formulated PsJN: stimulation of plant immunity + growth stimulation

GO enrichment (biological process) of DEGs of PsJN treated vs. untreated (Mock) at T6



GO enrichment (biological process) of DEGs of *Xf*DD-infected vs. non-infected under PsJN treatment at T6

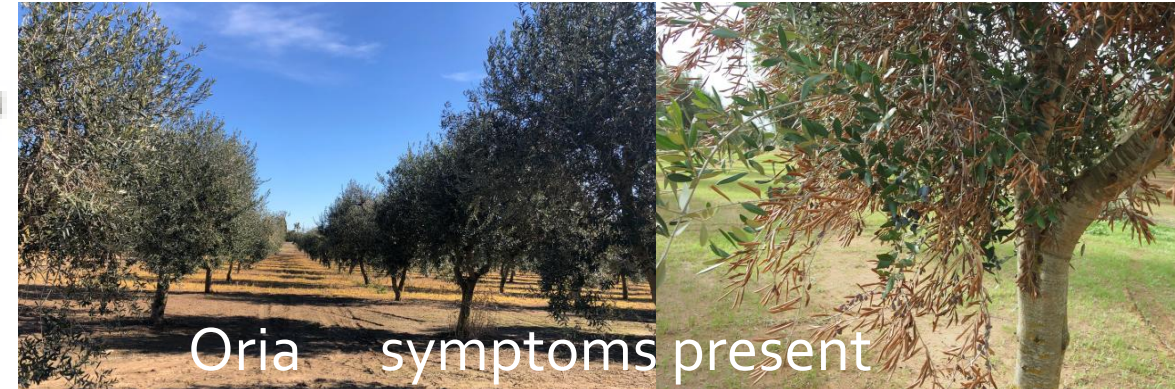


Curative trials 2022-2025



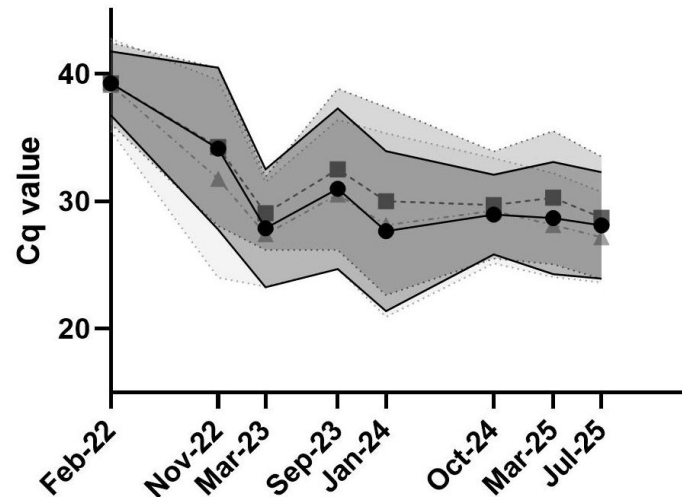
S. Vito dei N. planted 2022

- Formulated PsJN
 - Onion extract
 - Untreated
- 8 treatments/year



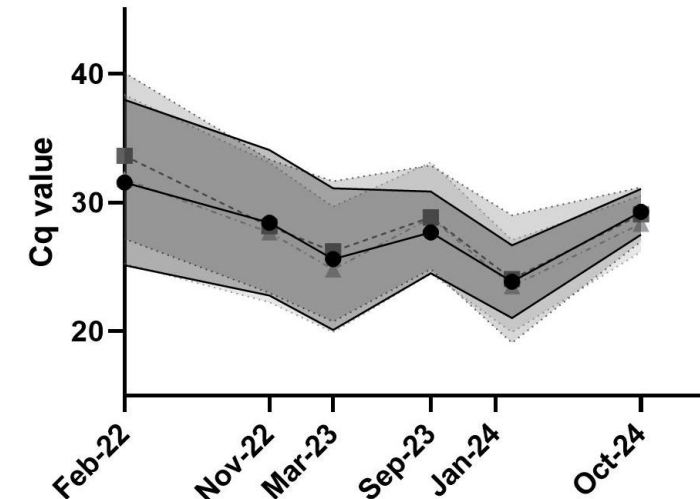
Oria symptoms present

- 4 randomized blocks/treatment 14 plants/block
- 56 plants/treatment, **168 plants**, Cellina di Nardò
- monitor Xylella qPCR and symptoms, every 6 months

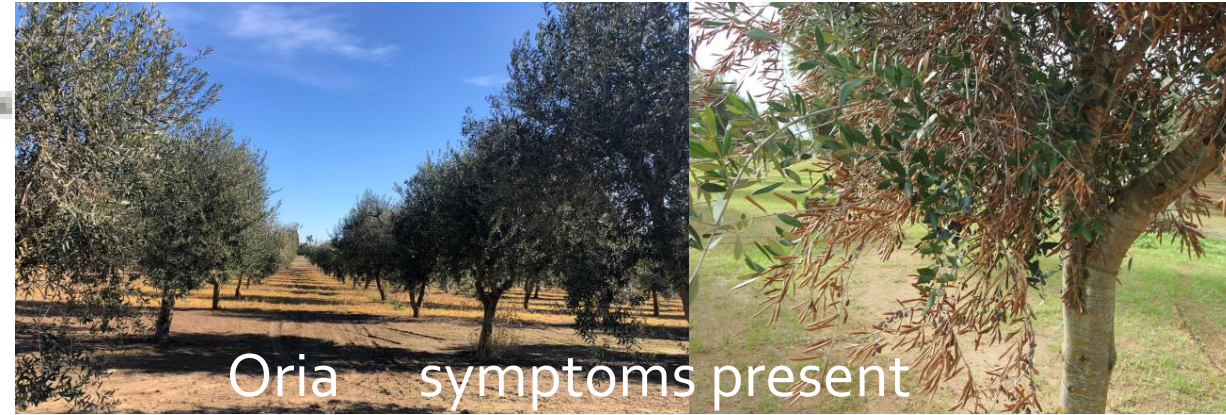


No eradication of Xylella

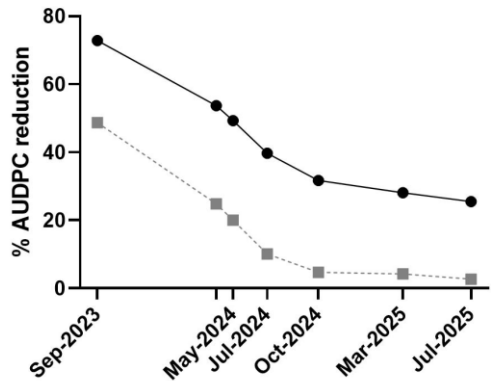
- 4 randomized blocks/treatment 10 plants/block
- 40 plants/treatment, **120 plants**, Cima di Melfi
- monitor Xylella qPCR and symptoms, every 6 months



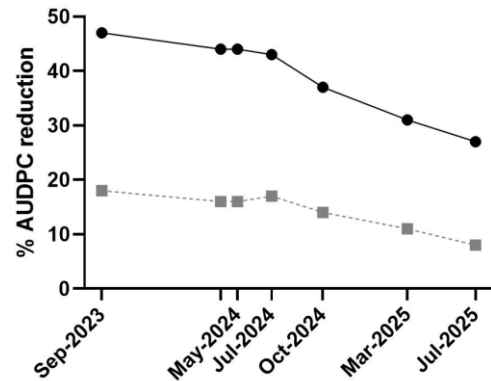
Curative trials 2022-2025



AUDPC Progress_Old symptoms

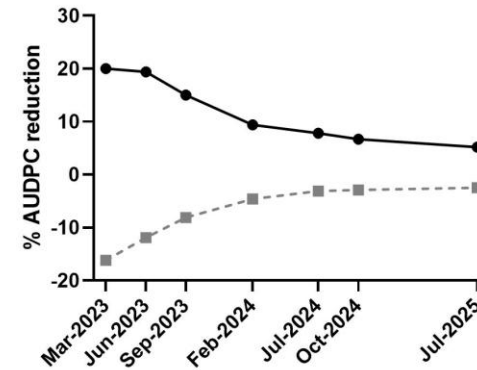


AUDPC Progress_New symptoms

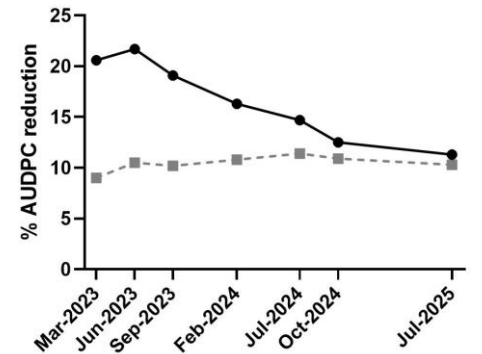


Disease mitigation
PsJN: 25.5-27.7%
Onion: 2.7-8.9%

AUDPC Progress_Old symptoms



AUDPC Progress_New symptoms

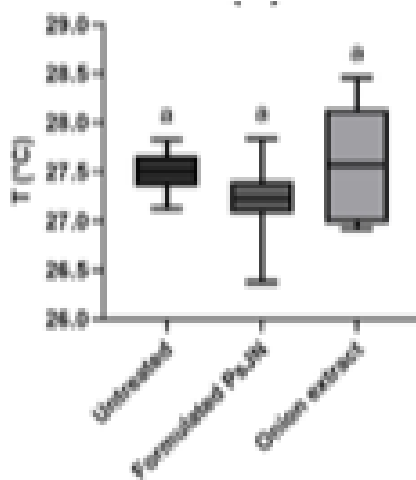
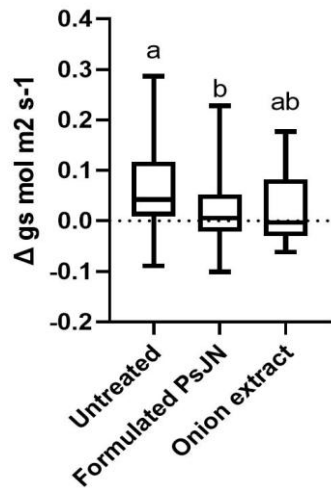
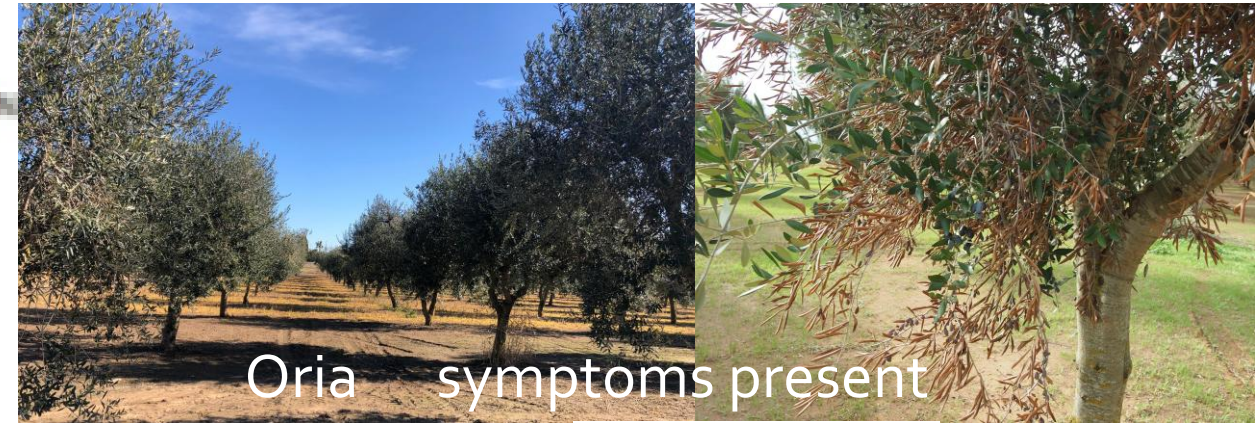


Disease mitigation
PsJN: 5.2-11.3%
Onion: 2.5-10.3%

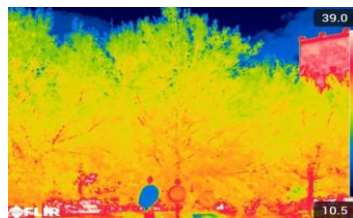
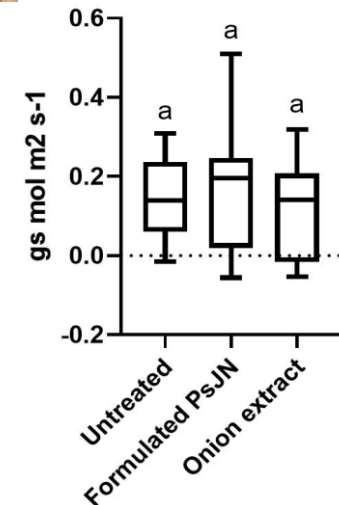
Curative trials Apulia 2022-2025



- Formulated PsJN
- Onion extract
- Untreated



- Stomatal conductance
- Canopy Temperature
- Better physiological conditions



Treated olives suffer less the drought stress of Xylella



Field trials Mallorca

Preventive trials



Formulated PsJN
Pseudomonas Pv8
Onion

8 treatments/year

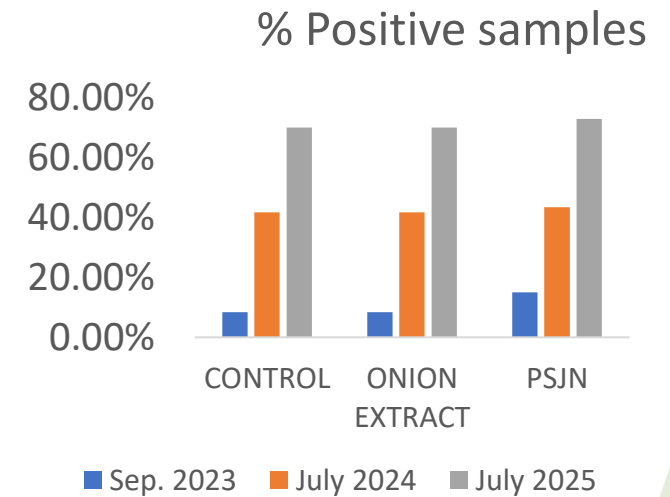
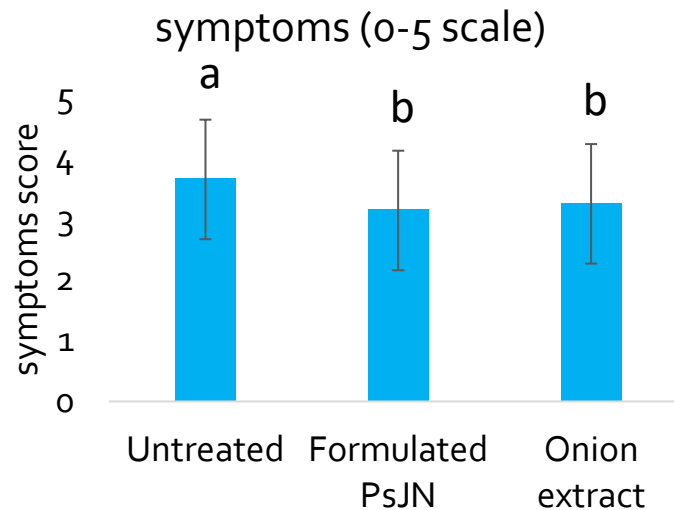


Productors Mallorquins de Fruit Secs

Curative trials

- Formulated PsJN
- Onion
- Lower symptoms severity of PsJN and Onion compared to the Untreated
- No difference of rate of infection

- No symptoms after years
- No difference between treatments
- Low rate of infections



Planted in 2022



Francavilla F.

Integrated Pest Management

(combining biopesticides to Xylella and Philaenus)

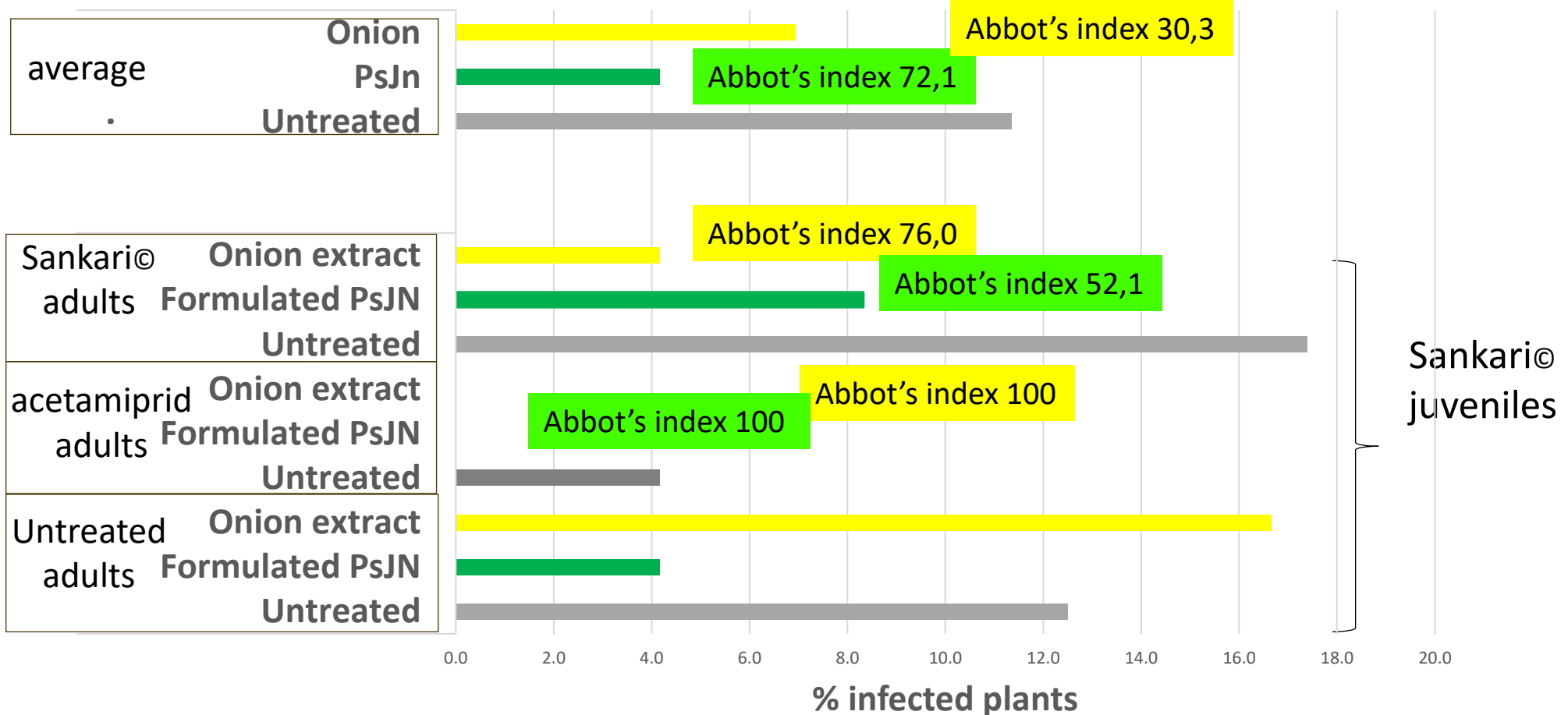
2022-2026

432 olives Ogliarola s.

Juveniles: Sankari® 2x treatments

Adults: Sankari® 11x treatments Sipcam 6x treatments

Form.PsJN and Onion: 8x treatments



Integrated Pest Management

(combining biopesticides to Xylella and Philaenus)

2022-2026

432 olives Ogliarola s.

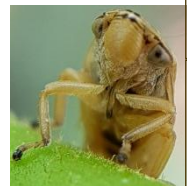
Juveniles: Sankari® 2x treatments

Adults: Sankari® 11x treatments Sipcam 6x treatments

Form.PsJN and Onion: 8x treatments



Squinzano



In a nutshell

- **Formulated PsJN:** slows down the spread of symptoms by up to **27.7 %** after 4 years as curative on young olive plants and improves plant vigour. Effects on olive and almonds.
- **Onion extract:** improves stomatal conductance, plant vigour, reduces appearance of new symptoms on olive and almond.
- **Sankari®.** Reduction of the juvenile and insect adults : **50%-54%**

Integrated Pest Management

- **Formulated PsJN** (% of infected plants reduced by **15.2% to 63.3% - Abbott's index**)
- **Sankari® (adults / juveniles)** 25%-40% reduction of infected plants
- Inconsistent results with Onion extract with IPM

Sankari® already on the market.



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

THANKS

Stéphane Compant

Pasquale Saldarelli

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AN INTEGRATED PROTOCOL TO MITIGATE OLIVE QUICK DECLINE SYNDROME (OQDS) CAUSED BY *XYLELLA FASTIDIOSA* SUBSP. *PAUCA* IN SOUTHERN ITALY

Del Grosso C.², Saponari M.², Saldarelli P.², Palmieri D.¹, Altamura G.^{2,3}, Abou Kubaa R.², Pastorini A.P.¹, De Curtis F.¹, Lima G.¹

1 - Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso 86100, Italy.

2 - Institute for Sustainable Plant Protection, National Research Council (CNR), Bari 70126, Italy.

3 - CRSFA-Centro Ricerca, Sperimentazione e Formazione in Agricoltura Basile Caramia, Locorotondo 70010, Italy.



Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026

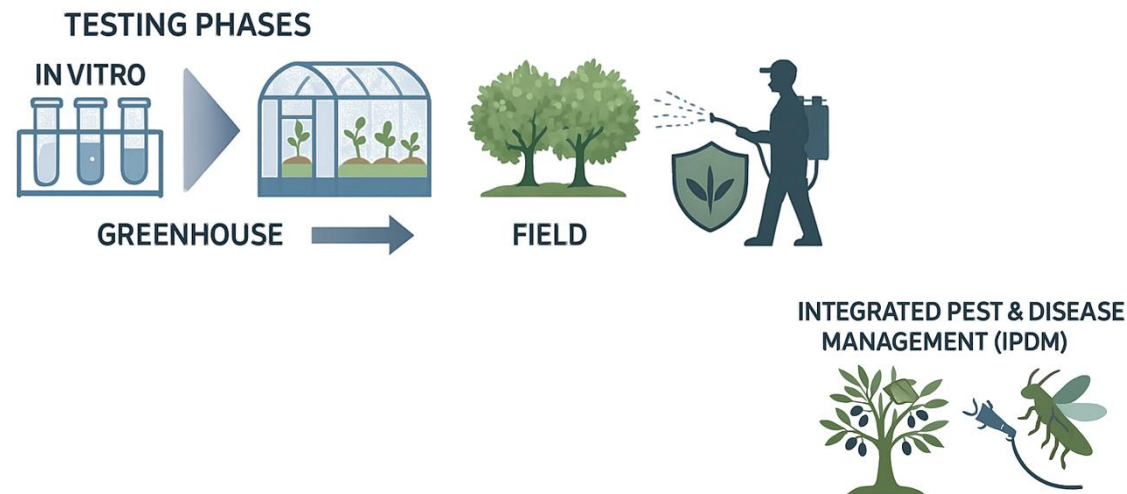


Objectives

- To evaluate the collateral antimicrobial activity of commercial fertilizers against *Xylella fastidiosa* subsp. *pauca* (*in vitro*, greenhouse, olive orchards)
- To measure effects on disease severity and bacterial populations
- To determine impact on olive yield and oil quality
- Validate the role of selected formulates within an IPDM strategy

Main tested formulates:

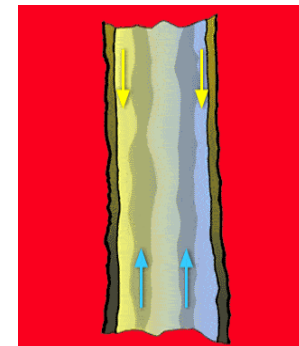
- Zinc phosphites (Kalex Zn®)
- Copper phosphites (Kalex Cu®)
- Bioavailable silicon (Siliforce®)



Products

➤ **Kalex Zn[®]**: Zinc phosphites

➤ **Kalex Cu[®]**: Copper phosphites

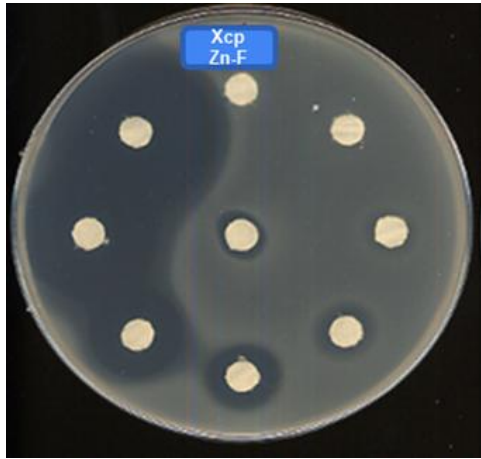


Fertilizers, promoting copper/zinc mobility (complete mobility) within the plant and stimulates, increasing plant's resistance to biotic and abiotic stress.

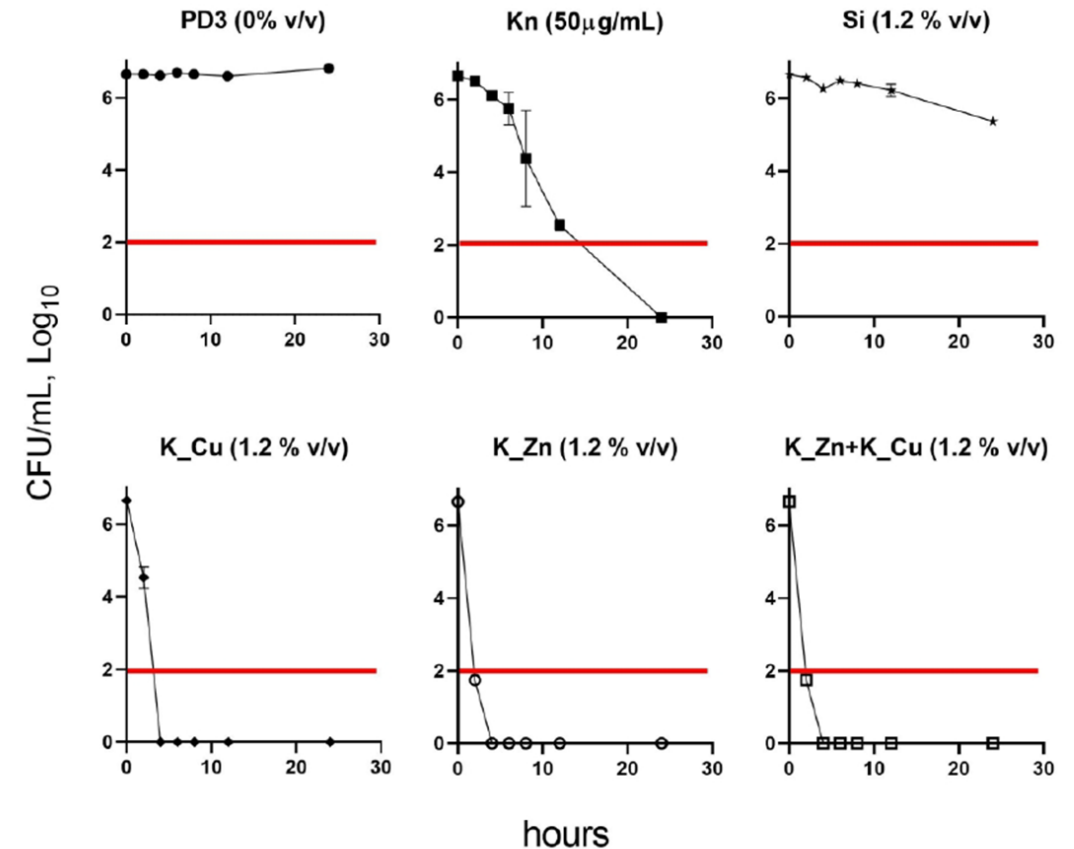
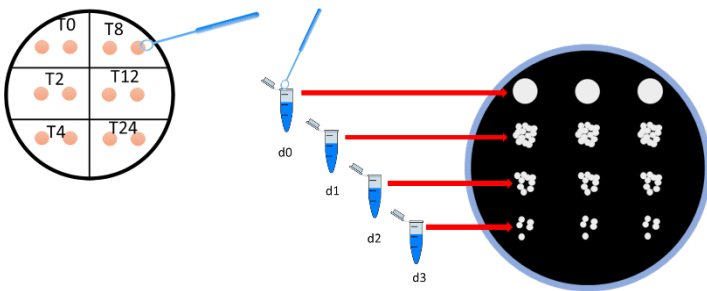
➤ **Siliforce[®]**: Bioavailable silicon (silicic acid), with low conc. molybdenum and zinc. Rep. to reinforce plant tissues and improve tolerance to both biotic and abiotic stress.

In vitro assays against *Xf* (Bactericidal Activity)

1- **Disk diffusion assay** to determine MIC



2 - **Time-kill contact assay** for bactericidal kinetics

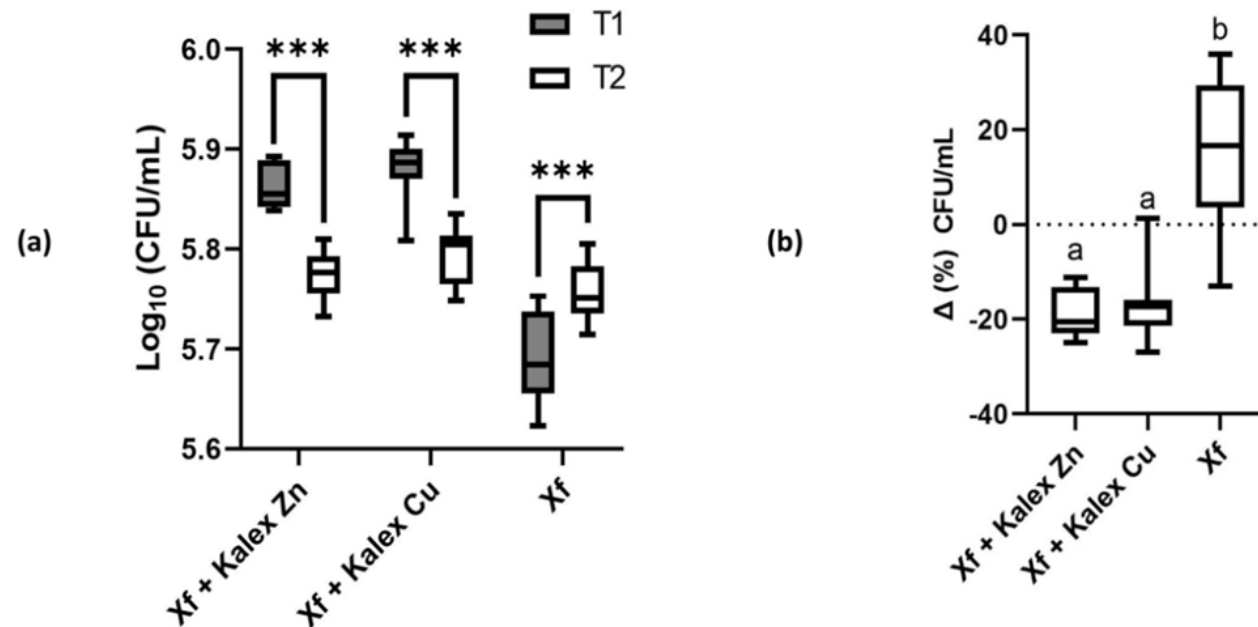


Disk diffusion and time-kill assays

- *stronger antibacterial activity* of **Kalex Zn** and **Kalex Cu**
- complete elimination of *Xfp* cells within 2–4 h of exposure
- direct bactericidal effects against *Xfp*

Greenhouse Trials

Graft-inoculated 'Ogliarola Salentina' olive plants treated monthly with Kalex Zn® or Kalex Cu® (the most effective compounds *in vitro*).

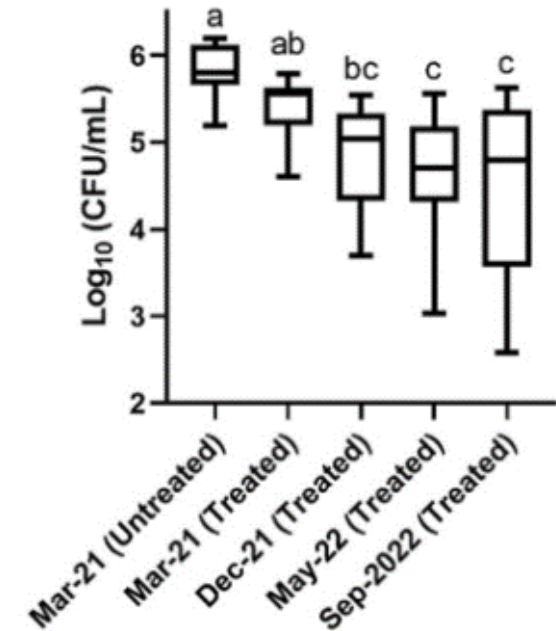
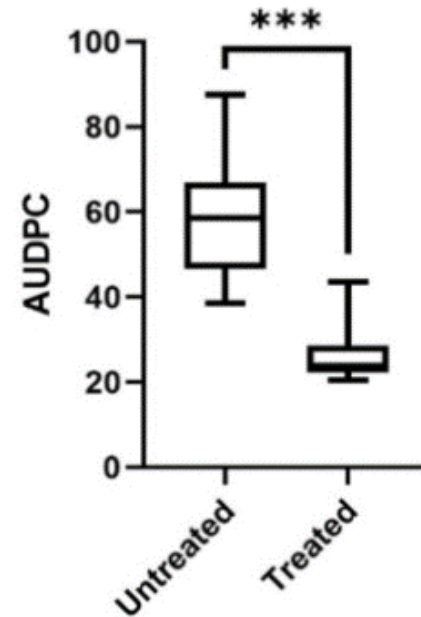
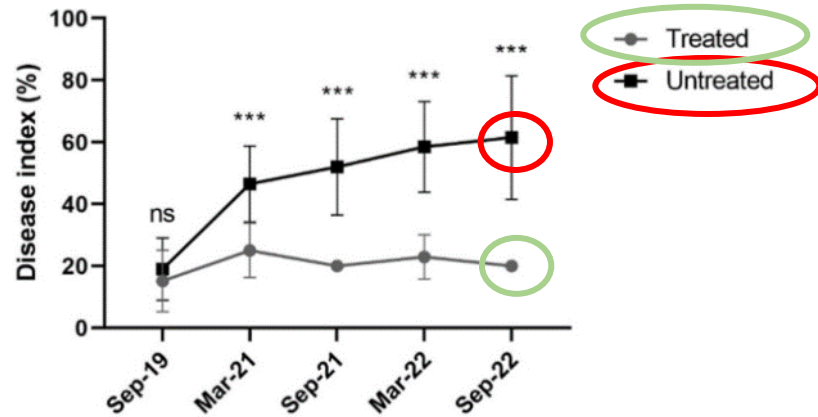


- **Kalex Zn** was the most effective product against *Xfp*, reducing disease severity ~50%
- Both, **Kalex Zn** and **Kalex Cu**, reduced the bacterial population

Field Trials



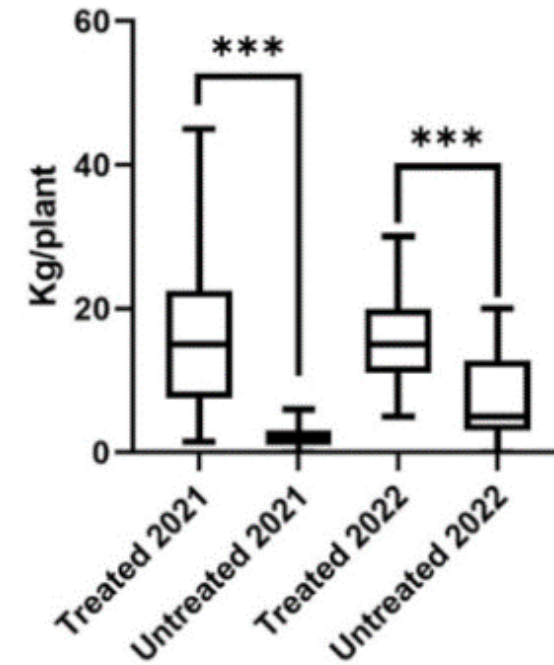
- Multi-year trials in a naturally infected orchard (cv. 'Cellina di Nardò')
- Five foliar sprays per year in an IPDM approach (soil tillage + yearly light pruning + vector control)
- Disease index, olive yield, *Xfp* population (via qPCR), and oil quality were assessed over a three-year period.



The protocol used

- Reduced Disease index ~50% vs the untr. control
- Reduced bacterial concentrations in treated trees

Olive yield and oil quality



Results

- Olive yield + ~ 50% (15.7 vs 7.5 kg/tree in untr. control)
- Improved oil quality (EVO quality and increased polyphenols levels).

2025, July

Conclusions

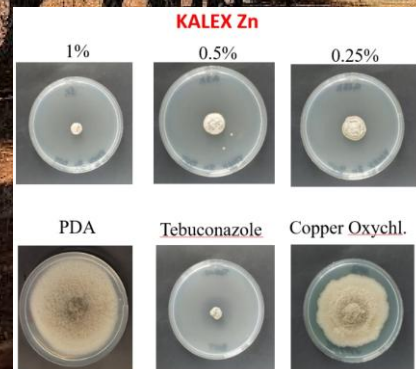
Selected fertilizer with strong collateral effect against *Xfp* can mitigate symptoms and bacterial load while improving yield and oil quality.

The use of these products within a broader IPDM promote long-term coexistence with the pathogen in the *Xfp*-infected area

Monumental trees

Tolerant/resistant var.

Untreated



Treated



Phenological Stage	VEGETATIVE ACTIVITY (March)	PRE-FLORING (April)	POST-FLORING / DRUPE DEVELOPMENT (May-June)	DRUPE VERGIL / MATURATION (July-August)	POSTHARVEST (October-November)	VEGETATIVE REST / PRUNING (February)
Management System	Monoculture	Monoculture	Monoculture	Monoculture	Monoculture	Monoculture
INTEGRATED OLIVECULTURE	Monoculture + Microbes and Microbes + Zn-Cu Phosphate	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists
INTEGRATED OLIVECULTURE	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists
INTEGRATED OLIVECULTURE	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists	Monoculture + Microbes and Microbes + Zn-Cu Phosphate + Antagonists

Soil tillage and weeds removal from February-March is an essential practice to reduce the vector population.
* Zn-Cu₂ Formulates in some olive groves are replaced, combined or alternated with 190P or Polyphenols.



Thank you for your attention!

Acknowledgements: Unimol, IPSP, Assays centers, olive growers, companies, etc.



Plant Pathology Unimol, Campobasso



IPSP CNR - Bari



Funding

INTEGROLIV (Project Code H33C22000860001) –

Minister of Agriculture, Food Sovereignty and Forests Italy (MASAF), D.M. n. 664829 29/12/20

(web site: www.integroliv.com)



Management of Olive Quick Decline Syndrome using Synthetic Bacterial Communities

L. Moll¹, L. Alonso-Villar¹, M. Anguita-Maeso^{1,2}, N. J. Flores-Duarte³, C. Haro¹, M. Román-Écija¹,
M. P. Velasco-Amo¹, C. Olivares-García¹, J. C. Rivas¹, D. Pérez-Bartivas¹, G. León-Ropero¹,
L. F. Arias-Giraldo¹, J. L. Trapero-Casas¹, F. Z. Benhalima¹, J. A. Navas-Cortés¹, B. B. Landa¹.

¹Department of Crop Protection, Institute for Sustainable Agriculture, Spanish National Research Council (IAS-CSIC), Córdoba, Spain

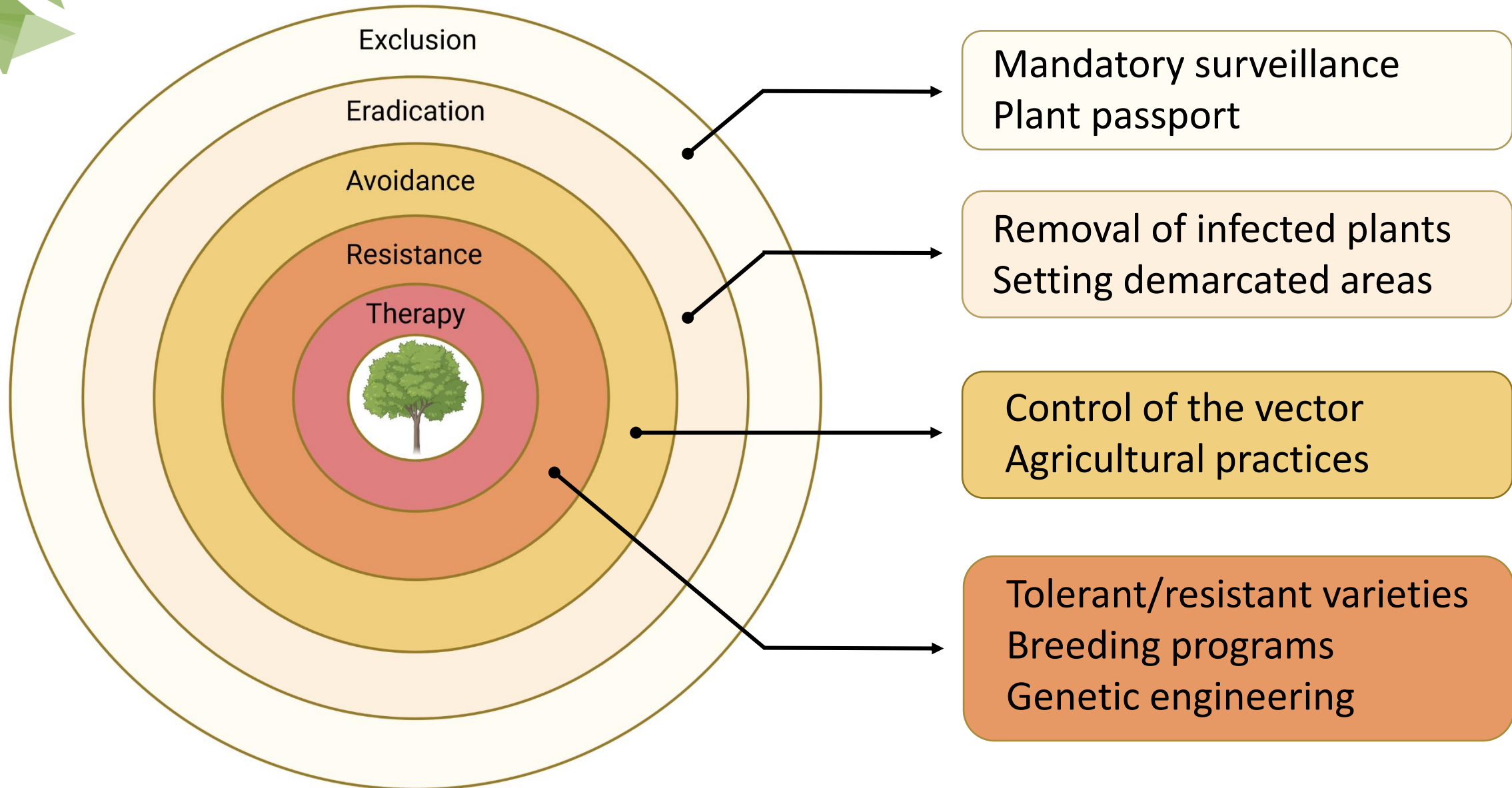
²University of North Carolina, Chapel Hill, USA

³Departamento de Microbiología y Parasitología, Universidad de Sevilla, Sevilla, Spain

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Management of *Xylella fastidiosa*

Integrated Disease Management (IDM)



Management of *X. fastidiosa*

Protection and therapy strategies

- No efficient treatments *in planta*
- Research is ongoing

EU plant health rules:
protecting plants, protecting lives.

Regulation (EU) 2016/2031



Reduction by 50% the use and risk of chemical pesticides by 2030



Reduction of approved active substances
Regulation of dosage

Directive 2009/128/EC (Sustainable Use of Pesticides)

Approved insecticides: neonicotinoids and pyrethroids
Antibiotics are banned in the agricultural sector



Resistance
emergence

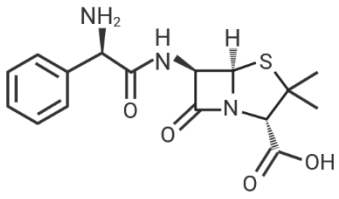


Need to find efficient and
eco-friendly alternatives

Protection and therapy strategies

Chemical control

Antibiotics



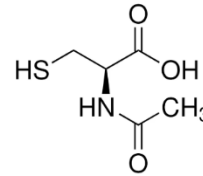
Amanifar, T. et al. 2016.
Phytopathol. Mediterr.

DSF application



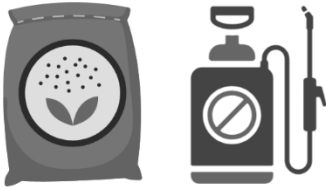
Horgan, C. et al. 2024.
Microorganisms.

N-Acetyl-L-cysteine (NAC)



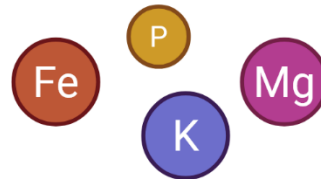
Picchi, S. C. et al. 2024.
Pest Manag. Sci.

Fertilizers



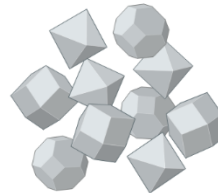
Del Grosso, C. et al. 2025.
Plant Dis.

Metal ions, or micronutrients



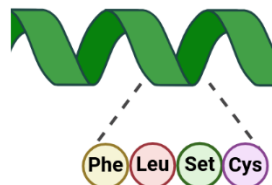
Orfei, B. et al. 2023.
Mol. Plant-Microbe Interact.

Nanocrystals



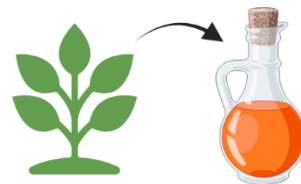
Tatulli, G. et al. 2024.
Phytopathology

Peptides



Moll, L. et al. 2025.
Plant Dis.

Plant derived compounds



Montesinos, L. et al. 2023.
Front. Plant Sci.

Biological control

Avirulent strains



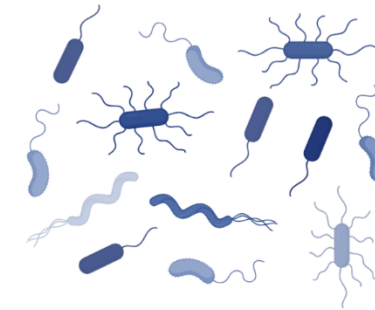
Hao, L. et al. 2017.
Mol. Plant Pathol.

Phages



Selles-Ribera, C. et al. 2026.
Plant Dis.

Bacteria and endophytes



Methylobacterium spp.

*Paraburkholderia
phytofirmans PsJN**

Curtobacterium flaccumfaciens

Other microorganisms

Carlucci, M. et al. 2025. *Chem. Biol. Technol. Agric.*
Manetsberger, J. et al. 2024. *Pest Manag. Sci.*
Lindow, S. et al. 2024. *Phytopathology*

Endophytic bacteria as biocontrol agents - SynComs

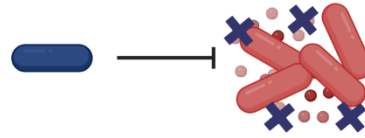
Biocontrol mechanisms

Direct mechanisms

Antimicrobial compounds/
hydrolytic enzyme production



Quorum sensing and quenching



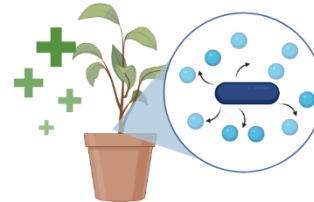
Competition for space
and nutrients



Activation of the Induced
Systemic Resistance (ISR)

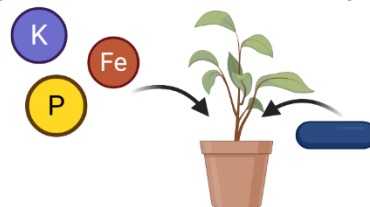


Plant hormone modulation

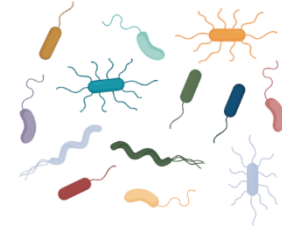


Indirect mechanisms

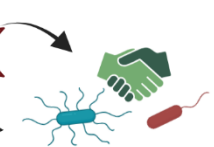
Improvement of nutrient uptake



Bacterial Synthetic Communities (SynComs)



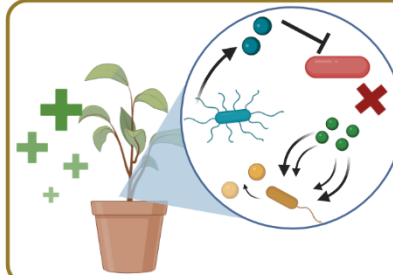
Higher niche
coverage



Resilience to
perturbations



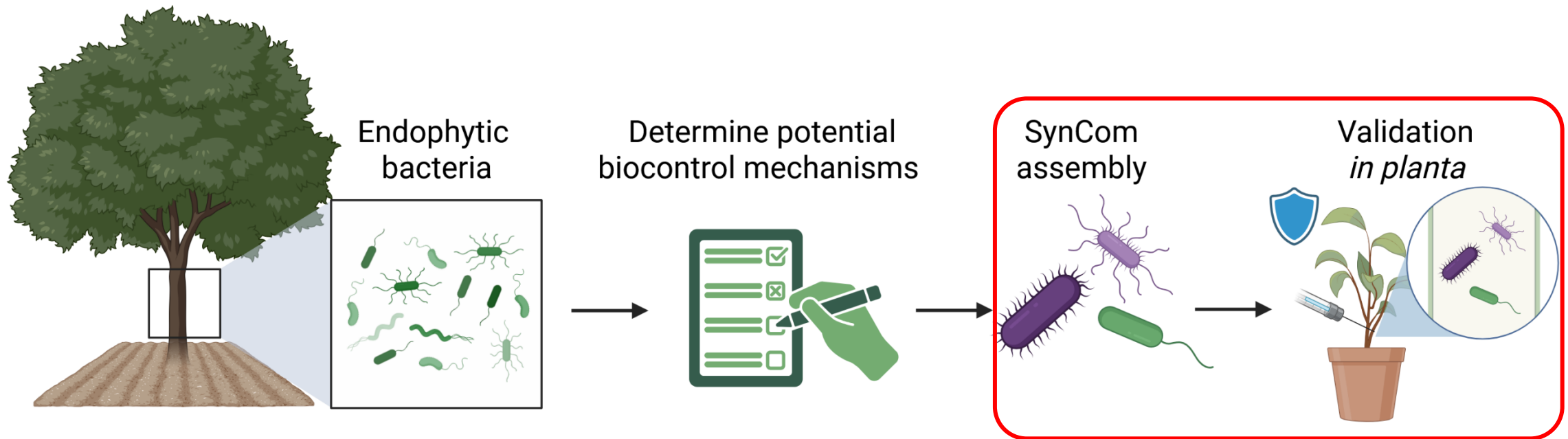
Higher stability



Multiple
mechanisms
of action

Objective

To characterize xylem-inhabiting bacteria with promising traits and design SynComs for the biocontrol of vascular diseases in olive trees



Overview of the *in vitro* experiments

302 olive-xylem strains

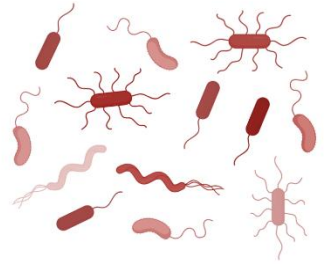
105 olive-xylem strains

26 strains

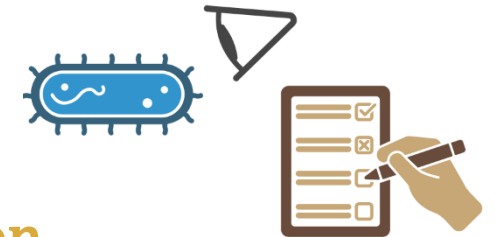
12 strains

5 strains

———— Collection of olive xylem-inhabiting isolated bacteria (BXOL) – Previous studies



———— A. Evaluation of direct and indirect biocontrol mechanisms



———— B. Carbon sources assimilation, biochemical sensitivity, *X. fastidiosa* inhibition

———— C. Compatibility assays



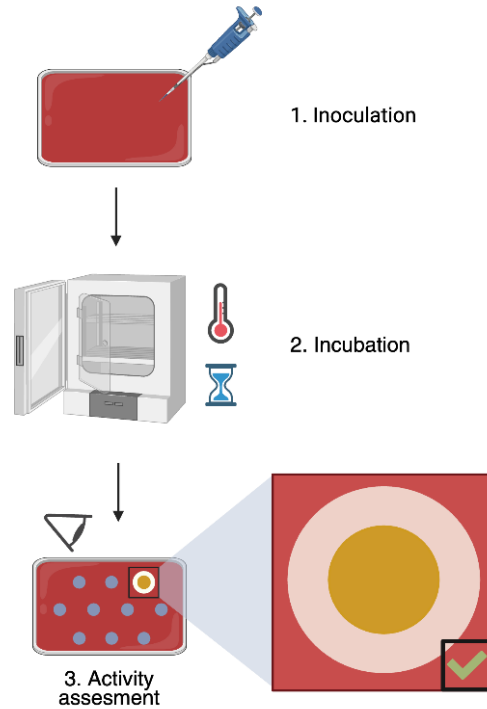
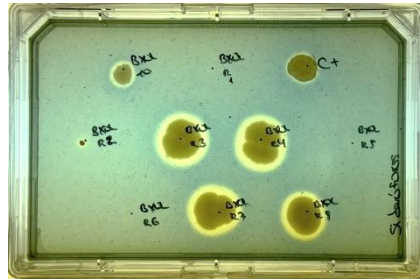
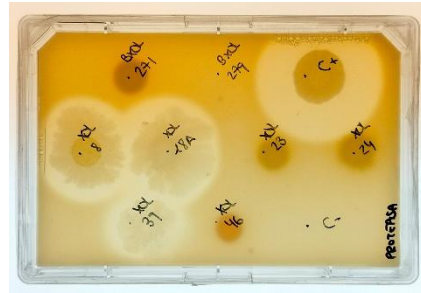
———— D. SynCom design



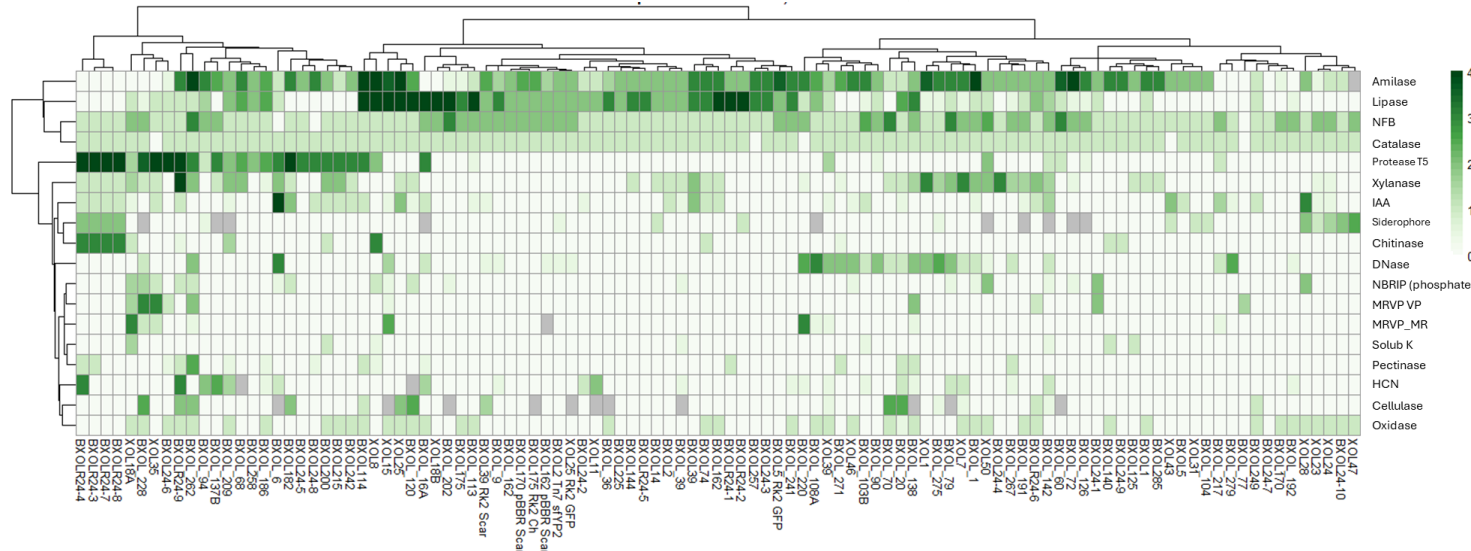
Evaluation of direct and indirect biocontrol mechanisms

302 olive-xylem strains

105 olive-xylem strains



Test	Culture medium	Reference
Nitrogen fixation	Nitrogen Fixing Bacteria medium	Döbereiner and Day, 1976
Phosphate Solubilization	NBRIP medium	Nautiyal, 1999
Potassium Solubilization	Alexandrov medium	Etesami et al., 2017
Siderophores	CAS assay	Schwyn and Neilands, 1987
IAA	NB + tryptophan	Naik et al., 2008
DNase	DNase agar	Scharlau, Spain
Amylase	Modified starch agar	Tindall et al., 2007
Catalase	-	Holt et al., 1994
Oxidase	-	Kovács, 1956
Cellulase	CMC solid medium	Elbeltagy et al., 2000
Lipase	Tween 80 solid medium	Prescott, 2002
Pectinase	AMA medium	McKay, 1988
Protease	Casein agar	Naik et al., 2008
Chitinase	Minimal medium + colloidal chitin	Murthy and Bleakley, 2012
Xylanase	Xylan source medium	Wood et al., 1988; Gong et al., 2012
Hydrogen cyanide (HCN)	King's B medium + glycine	Bano and Musarrat, 2003; Lorck, 1948
Methyl Red test	MRVP Broth	Britania Lab, Argentina
Voges Proskauer test	MRVP Broth	

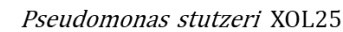


Presence or absence of specific activities was strain-dependent

26 strains

Some strains such as *B. safensis* XOL35, *Bacillus* sp. BXOL182 and *Sphingomonas aerolata* BXOL144 inhibited *X. fastidiosa* growth

71 carbon sources + 23
biochemical sensitivity assays



A-1 Negative Control C-	A-2 Dextrin	A-3 D-Maltose	A-4 D-Trehalose	A-5 D-Cellobiose	A-6 Gentiobiose	A-7 Sucrose	A-8 D-Turanose	A-9 Stachyose	A-10 Positive Control C+	A-11 pH 6	A-12 pH 5
B-1 D-Raffinose	B-2 α-D-Lactose	B-3 D-Melibiose	B-4 β-Methyl-D-Glucoside	B-5 D-Salicin	B-6 N-Acetyl-D-Glucosamine	B-7 N-Acetyl-β-D-Mannosamine	B-8 N-Acetyl-D-Galactosamine	B-9 N-Acetyl Neuraminic Acid	B-10 1% NaCl	B-11 4% NaCl	B-12 8% NaCl
C-1 α-D-Glucose	C-2 D-Mannose	C-3 D-Fructose	C-4 D-Galactose	C-5 3-Methyl Glucose	C-6 D-Fucose	C-7 L-Fucose	C-8 L-Rhamnose	C-9 Inosine	C-10 1% Sodium Lactate	C-11 Fusidic Acid	C-12 D-Serine
D-1 D-Sorbitol	D-2 D-Mannitol	D-3 D-Arabinol	D-4 myo-Inositol	D-5 Glycerol	D-6 D-Glucose-6-PO4	D-7 D-Fructose-6-PO4	D-8 D-Aspartic Acid	D-9 D-Serine	D-10 Macrolide	D-11 Rifamycin SV	D-12 Minocycline
E-1 Gelatin	E-2 Glycyl-L-Proline	E-3 L-Alanine	E-4 L-Arginine	E-5 L-Aspartic Acid	E-6 L-Glutamic Acid	E-7 L-Histidine	E-8 L-Pyroglutamic Acid	E-9 L-Serine	E-10 Lincomycin	E-11 Guanidine HCl	E-12 Niaproof 4
F-1 Pectin	F-2 D-Galacturonic Acid	F-3 L-Galactonic Acid Lactone	F-4 D-Gluconic Acid	F-5 D-Glucuronic Acid	F-6 Glucuronamide	F-7 Mucic Acid	F-8 Quinic Acid	F-9 D-Saccharic Acid	F-10 Vancomycin	F-11 Tetrazolium Violet	F-12 Tetrazolium Blue
G-1 p-Hydroxy-Phenylacetic Acid	G-2 Methyl Pyruvate	G-3 D-Lactic Acid Methyl Ester	G-4 L-Lactic Acid	G-5 Citric Acid	G-6 α-Keto-Glutaric Acid	G-7 D-Malic Acid	G-8 L-Malic Acid	G-9 Bromo-Succinic Acid	G-10 Nalidixic Acid	G-11 Lithium Chloride	G-12 Potassium Tellurite
H-1 Tween 40	H-2 γ-Amino-Butyric Acid	H-3 α-Hydroxy-Butyric Acid	H-4 β-Hydroxy-DL-Butyric Acid	H-5 α-Keto-Butyric Acid	H-6 Acetoacetic Acid	H-7 Propionic Acid	H-8 Acetic Acid	H-9 Formic Acid	H-10 Aztreonam	H-11 Sodium Butyrate	H-12 Sodium Bromate

The diagram illustrates the experimental workflow for *X. fastidiosus* (Xf) infection. It begins with Xf stocks stored at -80 °C. The workflow branches into two methods: 1) Direct inoculation into R2A medium, which takes 2 days. 2) Lawn inoculation, which involves a 7-day incubation followed by a 5-day incubation, totaling 12 days. Both methods result in a petri dish showing various colored colonies (orange, yellow, pink, white, brown, grey).

X. fastidiosa
inhibition
assay

Time (days)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
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Compatibility assays

302 olive-xylem strains

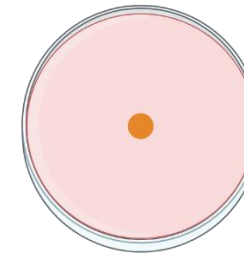
105 olive-xylem strains

26 strains

12 strains

Dual culture experiment

Background: Strain A
Spot: Strain B



Compatible strains
(growth)



Incompatible strains
(no growth / inhibition)

Strain	BXOL 2	BXOL 39	BXOL 162	BXOL 200	BXOL 215	BXOL 249	XOL 25	XOL 28	XOL 35	XOL 43	BXOL 170	BXOL 175
BXOL 2	+	-	+	+	+	+	+	+	+	+	-	+
BXOL 39	+	+	++	±	+	±	±	++	±	++	-	+
BXOL 162	+	+	+	+	+	+	+	+	-	+	-	-
BXOL 200	++	+	+	+	+	-	+	++	±	±	--	+
BXOL 215	--	--	--	--	+	--	+	--	-	--	--	-
BXOL 249	+	-	-+	--+	+	+	++	+	-	±	-	+
XOL 25	-+	+	+	+	+	-	+	+	+	+	-	-
XOL 28	+	±	±	-	--	±	+	+	-	-	-	-
XOL 35	-	±	+	±	-	±	+	±	+	-	--	-
XOL 43	+	+	+	+	-	+	+	+	--	+	--	+
BXOL 170	+	+	+	+	+	+	+	+	±	+	+	+
BXOL 175	++	++	+	+	+	+	+	+	--	+	-	+

>60% of total interactions
exhibited mutual tolerance or
growth promotion

SynCom design and assembly

302 olive-xylem strains

105 olive-xylem strains

26 strains

12 strains

5 strains

SynCom	Strain 1	Strain 2	Strain 3
1	<i>Bacillus safensis</i> XOL35	<i>Novosphingobium fluoreni</i> BXOL39	<i>Variovorax</i> sp. BXOL162
2	<i>Rhizobium pusense</i> / <i>Agrobacterium pusense</i> XOL28	<i>Methylobacterium radiotolerans</i> XOL43	<i>Novosphingobium fluoreni</i> BXOL39

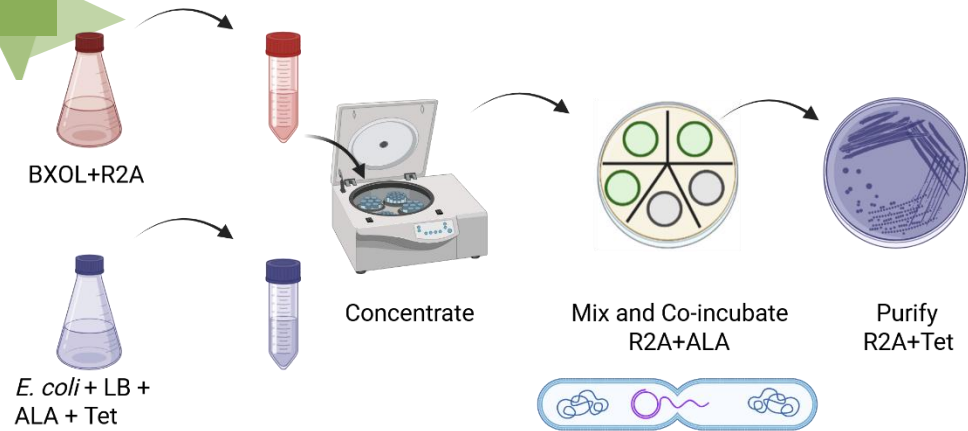
	Strain	P solubilization	Siderophores	IAA	Nitrogen fixation	K solubilization	DNase	Amylase	Cellulase	Pectinase	Protease	Chitinase	Lipase	Xylanase	HCN	Catalase	Oxidase	Methyl red test	Voges-Proskauer	Xf inhibition	Vd inhibition
SC1	XOL35																				
	BXOL39																				
	BXOL162																				
SC2	XOL28																				
	XOL43																				
	BXOL39																				

Test strain	T1 XOL35	T2 BXOL39	T3 BXOL162	T1+T2	T2+T3	T1+T3	T1+T2+T3
Background strain							
B1 XOL35	+	+	+	+	+	+	+
B2 BXOL39	+	+	+	+	+	+	+
B3 BXOL162	+	+	+	+	+	+	+ by intersection
Test strain	T4 XOL28	T5 XOL43	T6 BXOL39	T4+T5	T5+T6	T4+T6	T4+T5+T6
Background strain							
B4 XOL28	+	-	+	+	+	+	+ by intersection
B5 XOL43	++	++	++	+	+	+	+
B6 BXOL39	+	++	+	+	++	+	+ by intersection

SynCom *in vitro* performance vs *X. fastidiosa* was confirmed to be consistent with the activity shown by the individual strains

SynCom design and assembly

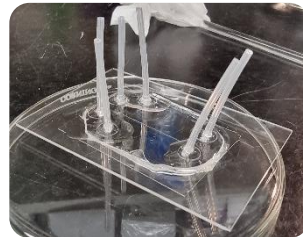
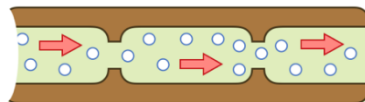
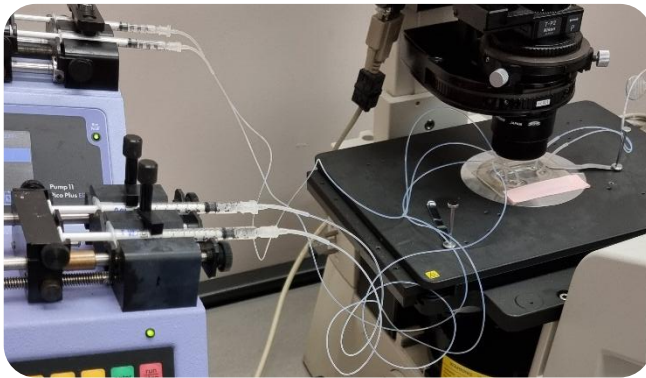
Obtaining fluorescent BXOL strains → Conjugation



SC1

XOL35
(GFP)

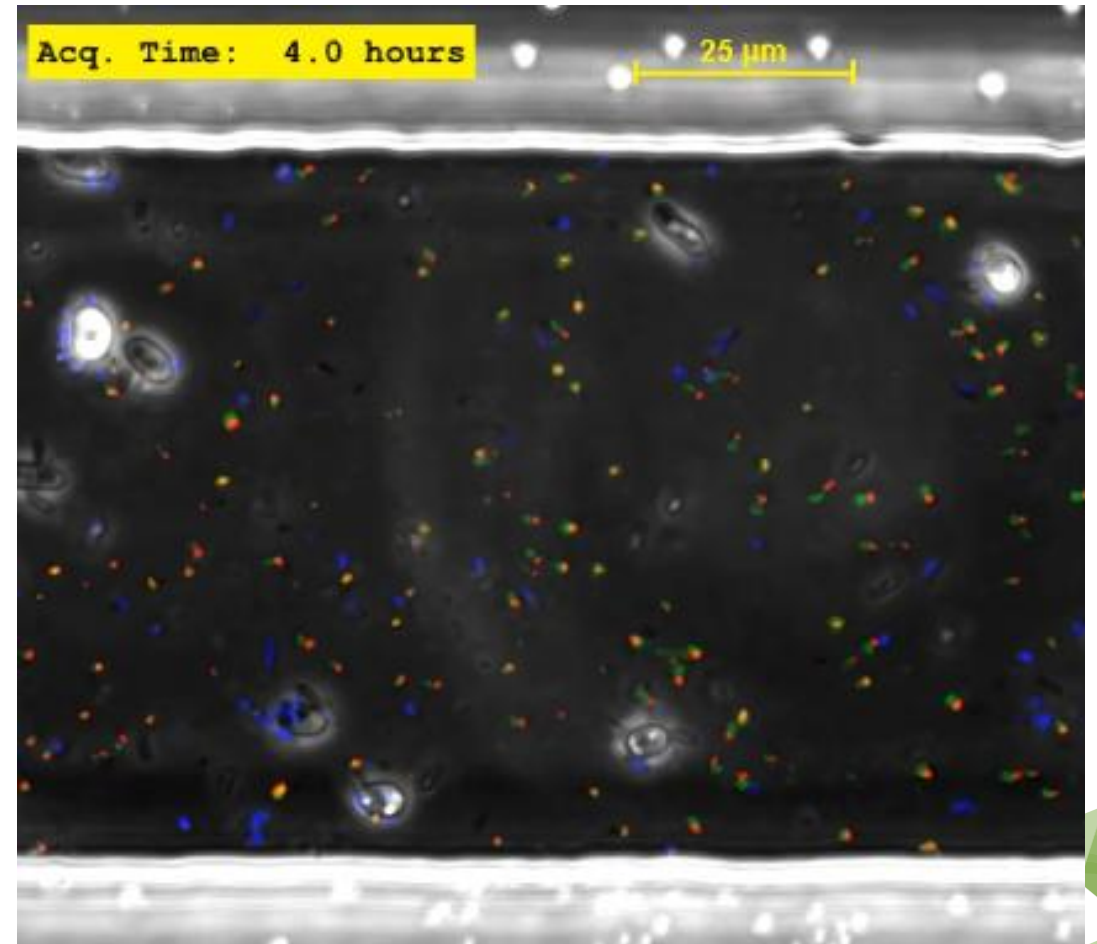
Confirmation of compatibility → Microfluidics



BXOL162
(mScarlet)

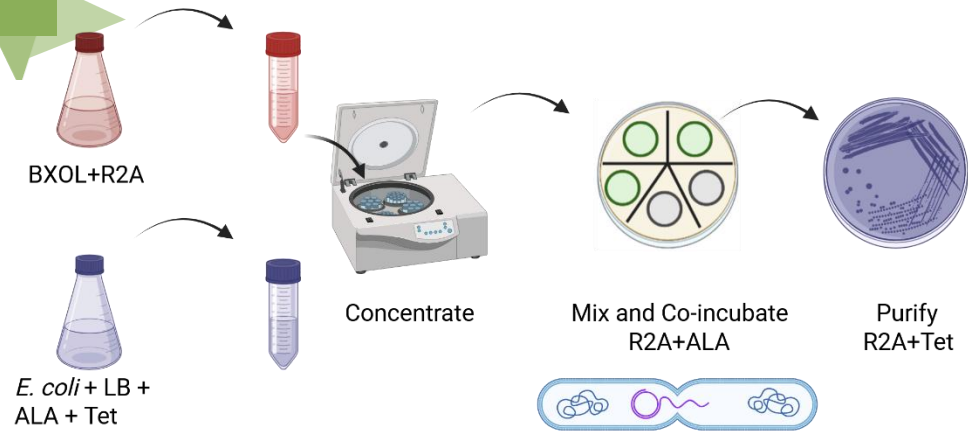
BXOL39
(mTagBFP)

All strains in both SC were able to grow together

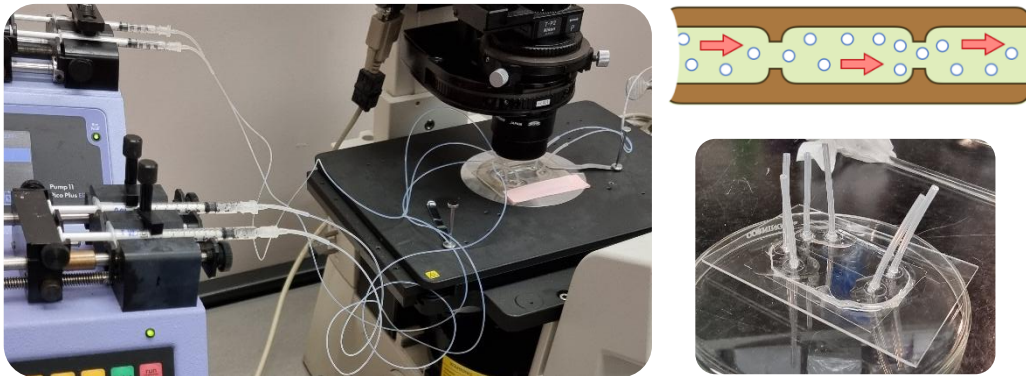


SynCom design and assembly

Obtaining fluorescent BXOL strains → Conjugation



Confirmation of compatibility → Microfluidics



All strains in both SC were able to grow together

SC1

XOL35
(GFP)

0h

0.25%

24h

4.4%

BXOL162
(mScarlet)

0.18%

0.81%

BXOL39
(mTagBFP)

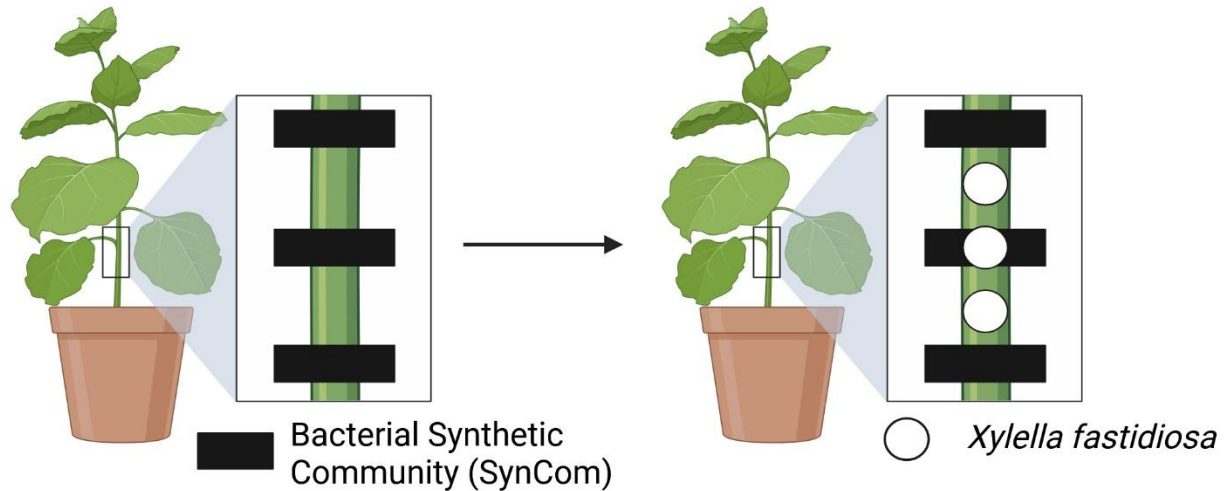
0.73%

1.87%

Validation *in planta* – Overall methodology

Model plant: *Nicotiana benthamiana*

Target host: *Olea europaea*



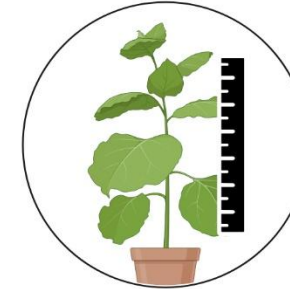
SC1 or SC2
OD₆₀₀=0.3 in R2A broth
Aprox. 10⁸ CFU/mL
Strains ratio = 1:1:1
10 µL x 3 injections

X. fastidiosa subsp. *pauca* DeDonno
OD₆₀₀=1 in PD3 broth
Aprox. 10⁸ CFU/mL
10 µL x 3 injections

Disease severity



Shoot system height



Population levels (qPCR)



Leaf physiological parameters (DUALEX)



Fresh weight



SynCom inoculation

Pathogen inoculation

Sampling and monitoring

Start of experiment

3 days after

Depending on host/pathogen

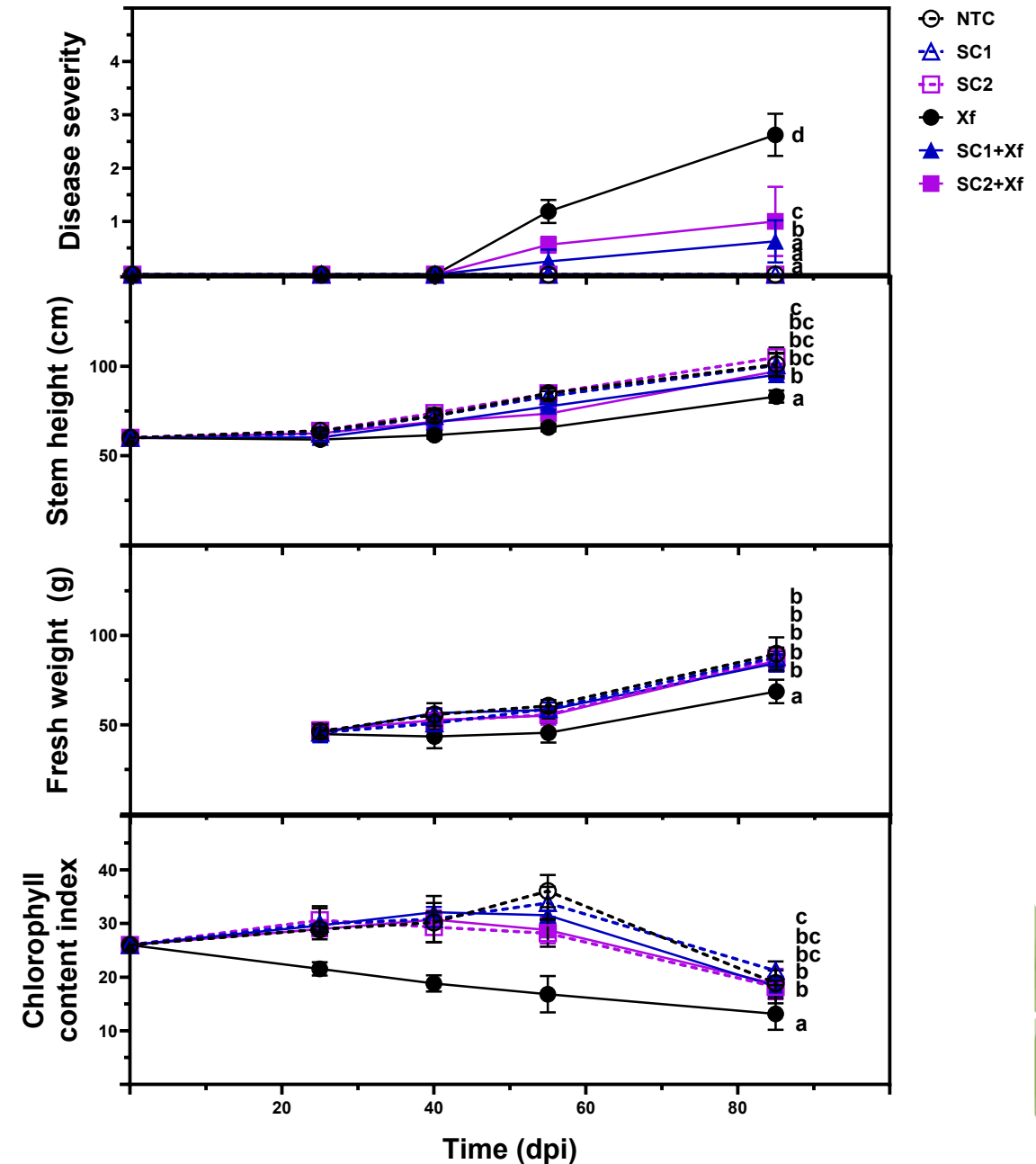
End of experiment

Validation *in planta* – *N. benthamiana* + *X. fastidiosa* (Xf)

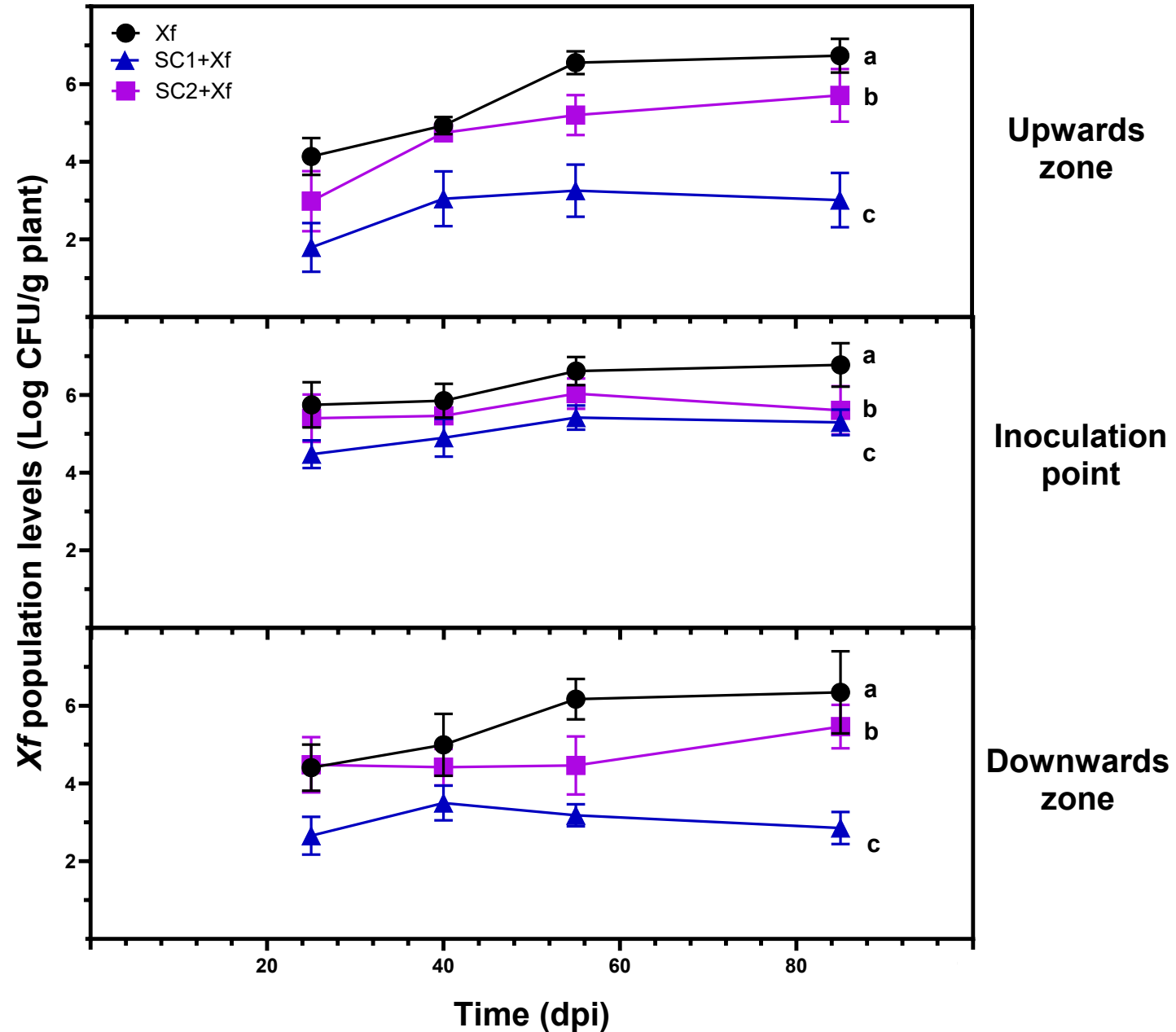
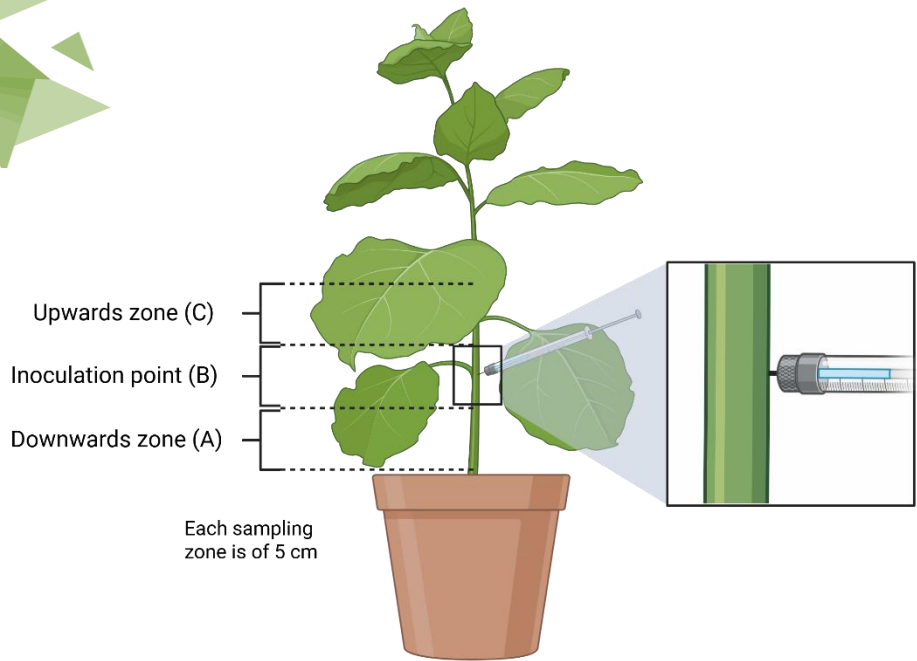


85 dpi	Xf	SC1+Xf	SC2+Xf
Disease severity	2.6	↓ 77%	↓ 62%
Stem height (cm)	83	↑ 23%	↑ 25%
Fresh weight (g)	68.6	↑ 14%	↑ 17%
Chlorophyll content	13.1	↑ 38%	↑ 42%

- Plants treated with SC1 and SC2 and infected by *Xf*:
 - Showed a reduction in disease development
 - Showed a growth similar to NTC



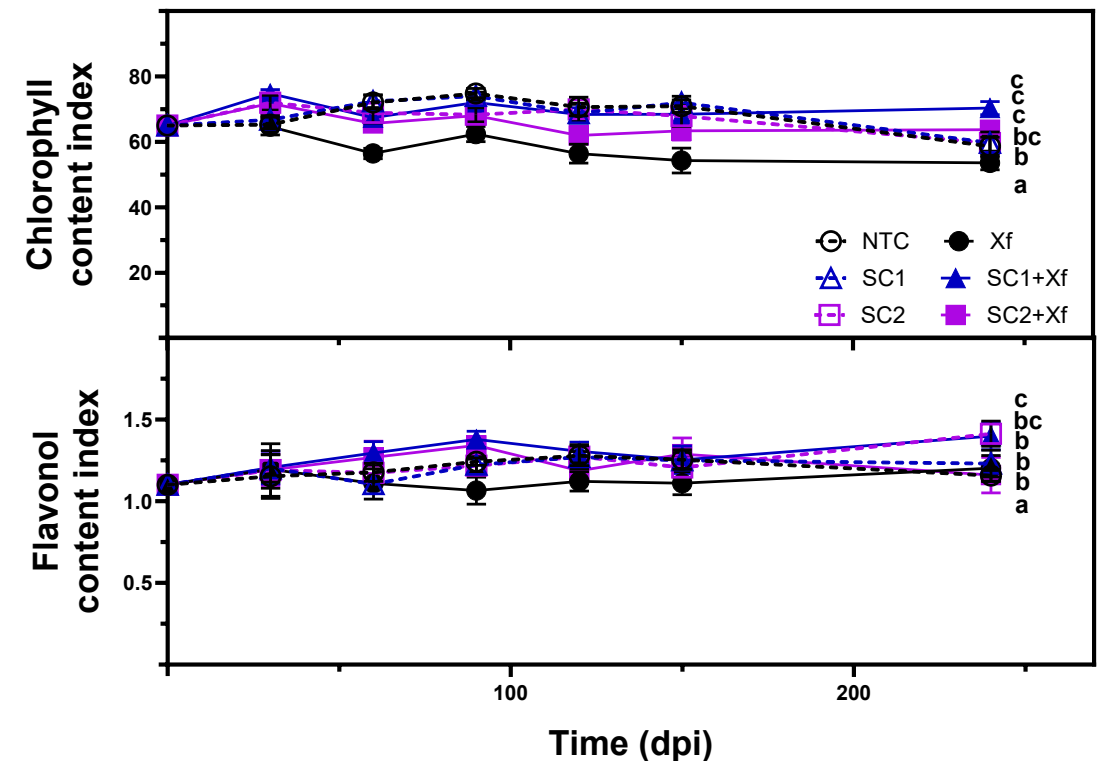
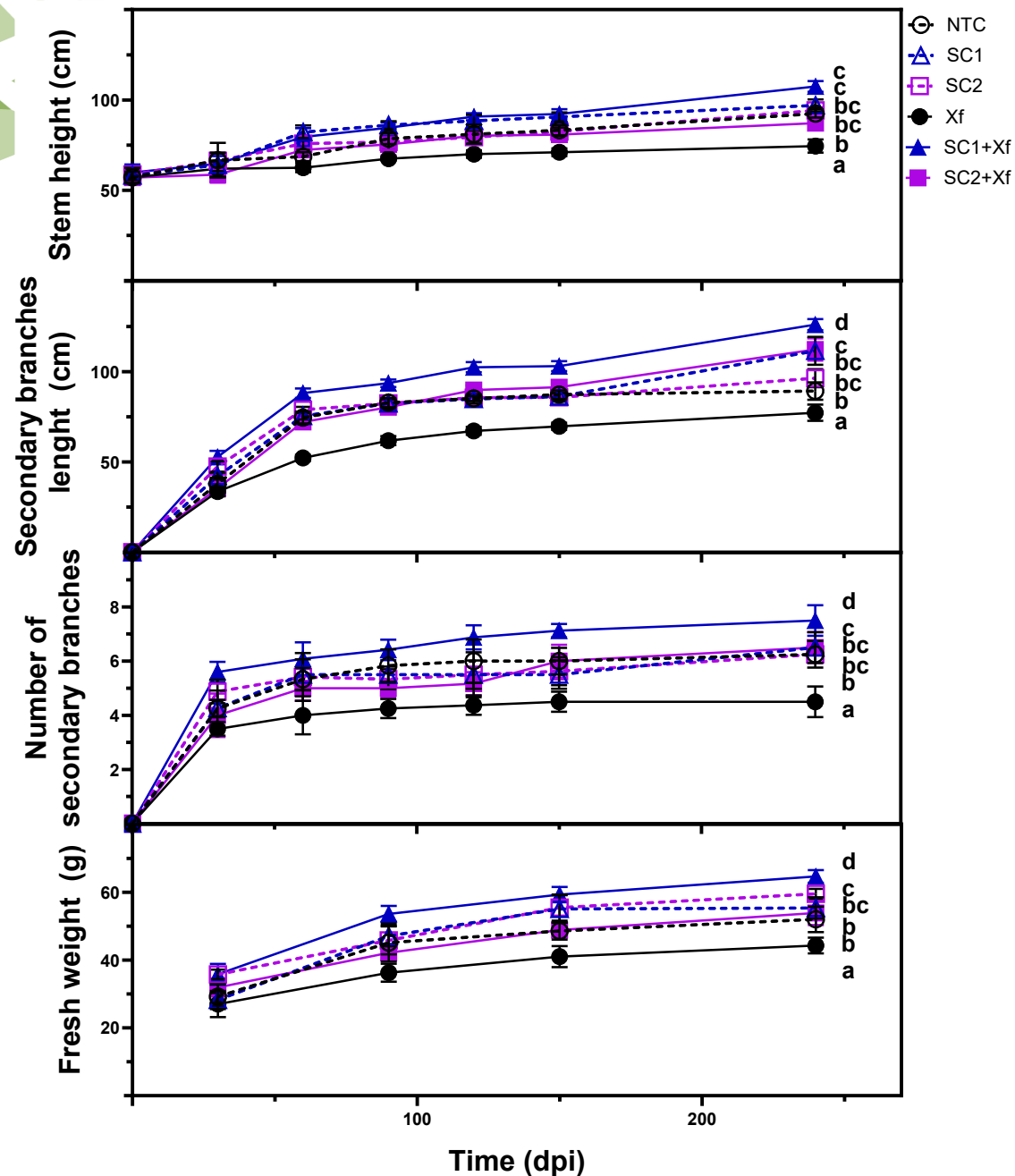
Validation *in planta* – *N. benthamiana* + *X. fastidiosa* (Xf)



55 dpi	Xf	SC1+Xf	SC2+Xf
Upwards zone	6.6	↓3.3 log	↓1.4 log
Inoculation point	6.6	↓1.2 log	↓0.5 log
Downwards zone	6.2	↓3 log	↓1.7 log

SC1 caused the highest population level reduction in most of the stem sections

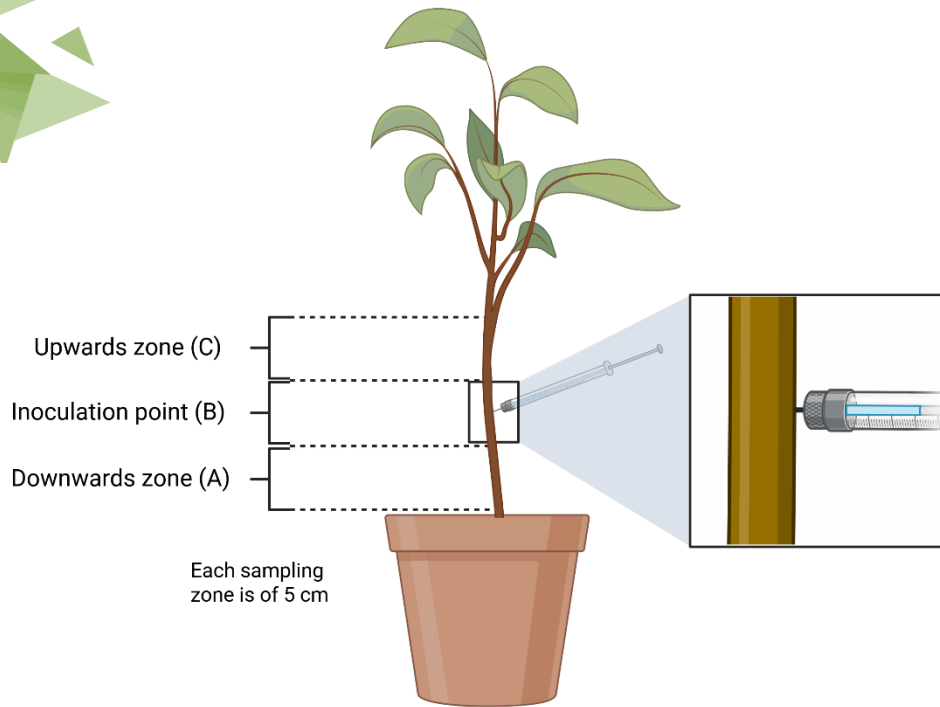
Validation *in planta* – *O. europaea* + *X. fastidiosa* (Xf)



240 dpi	Xf	SC1+Xf	SC2+Xf
Stem height (cm)	74.5	↑ 44%	↑ 17%
Nº branches	4.3	↑ 66%	↑ 44%
Sum lenght branches (cm)	77.3	↑ 63%	↑ 45%
Fresh weight (g)	44.3	↑ 46%	↑ 22%
Chlorophyll content	53.6	↑ 31%	↑ 19%
Flavonol content	53.6	↑ 16%	0%

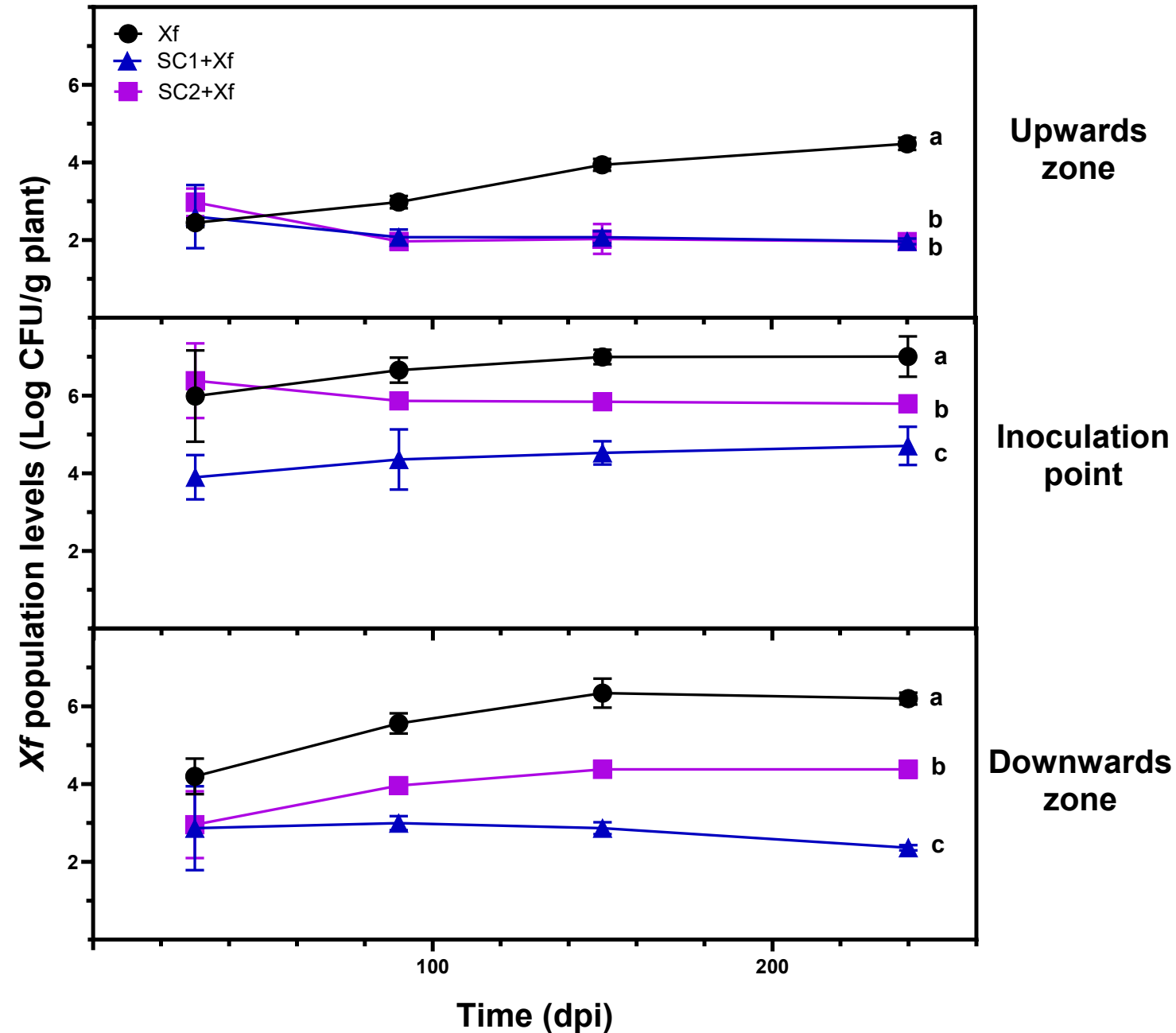
SC1 showed the best results

Validation *in planta* – *O. europaea* + *X. fastidiosa* (Xf)



240 dpi	Xf	SC1+Xf	SC2+Xf
Upwards zone	4.5	↓2.5 log	↓2.5 log
Inoculation point	7	↓2.3 log	↓1.2 log
Downwards zone	6.2	↓3.8 log	↓1.8 log

SC1 caused the highest *Xf* population level reduction in all analyzed sections



Experiments on field



31 partners
17 countries



Experiments are being carried out on field for the management of *Xf* with SynComs (Italy and Balearic Islands)
<https://bexylproject.org/>



SynCom development
and application



SynCom production
in bioreactors



Low pressure
endotherapy devices



Italy on field support
(olive)



IRFAP

Institut de Recerca i Formació
Agroalimentària i Pesquera
de les Illes Balears

Balearic Islands on field
support (almond)



Olive



Almond

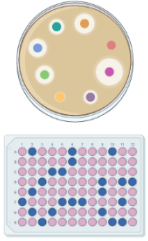


Disease development and
Population of *Xf* and SynCom
strains will be monitored

Conclusions



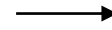
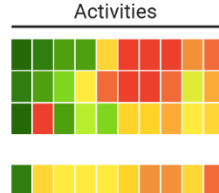
BXOL collection
(>300 strains)



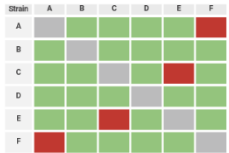
Biocontrol
mechanisms

Metabolic
profile

Strains
A
B
C
...
Z



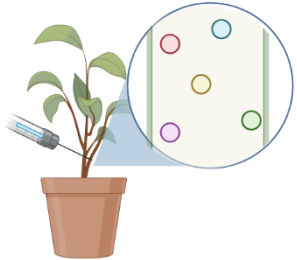
- 105 strains were characterized
- Biocontrol-associated traits were strain-dependent



Compatibility
and activity
assessment



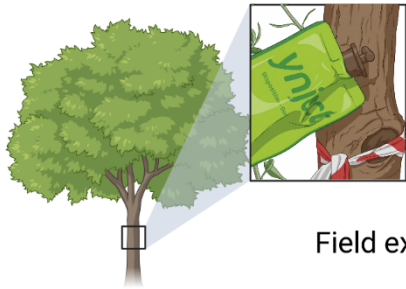
- Selected strains showed compatibility
- Two SynComs were designed
- All strains grew together and retained activity



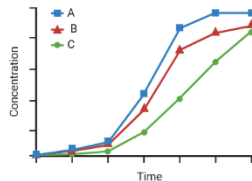
Population fitness and plant
fitness assessment



- Both SynComs showed activity against *Xf in planta* and improved plant fitness
- SC1 showed the best results



Field experiments



Healthy

Sick

?



- Field experiments are being carried out in Mallorca and Italy for the management of *Xf*

Acknowledgements



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- MCIN/AEI/10.13039/501100011033 (PID2020-114917RB-100)
- MICIU/AEI/10.13039/501100011033 “European Union Next Generation EU/PRTR” (TED2021-130110BC41)
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- Qualifica Project QUAL21_023 IAS

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Some images were generated with BioRender.com



Funded by
the European Union



Management of Olive Quick Decline Syndrome using Synthetic Bacterial Communities

L. Moll¹, L. Alonso-Villar¹, M. Anguita-Maeso^{1,2}, N. J. Flores-Duarte³, C. Haro¹, M. Román-Écija¹,
M. P. Velasco-Amo¹, C. Olivares-García¹, J. C. Rivas¹, D. Pérez-Bartivas¹, G. León-Ropero¹,
L. F. Arias-Giraldo¹, J. L. Trapero-Casas¹, F. Z. Benhalima¹, J. A. Navas-Cortés¹, B. B. Landa¹.

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²University of North Carolina, Chapel Hill, USA

³Departamento de Microbiología y Parasitología, Universidad de Sevilla, Sevilla, Spain

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Thank you for
your attention!



Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026

From Detection to Control: Integrating Aptamer-based Diagnostics with Phage and Antimicrobial Peptide Strategies Against *Xylella fastidiosa*

Toufic ELBEAINO

CIHEAM Bari, Italy

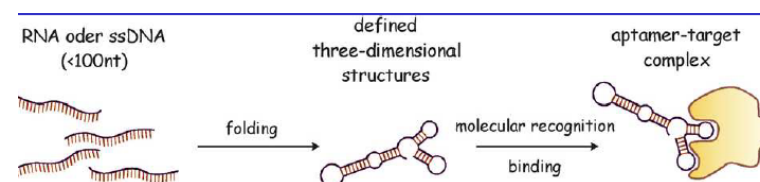


REGIONE PUGLIA

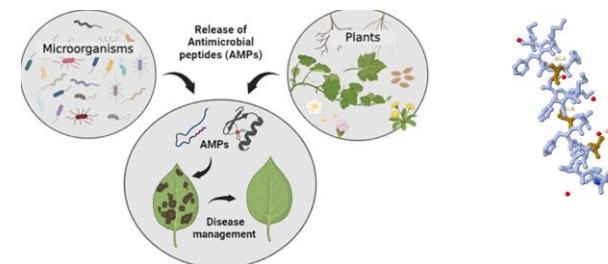
Aims and perspectives:

Develop field-deployable diagnostics and sustainable biological control strategies for *Xylella fastidiosa* in olive trees in Italy

➡ **Aptamers:** stable, specific and easy to modify; ideal for rapid, low-cost field diagnostics



➡ **Antimicrobial Peptides (AMPs):** natural molecules that suppress *Xylella* growth and support low-residue disease management



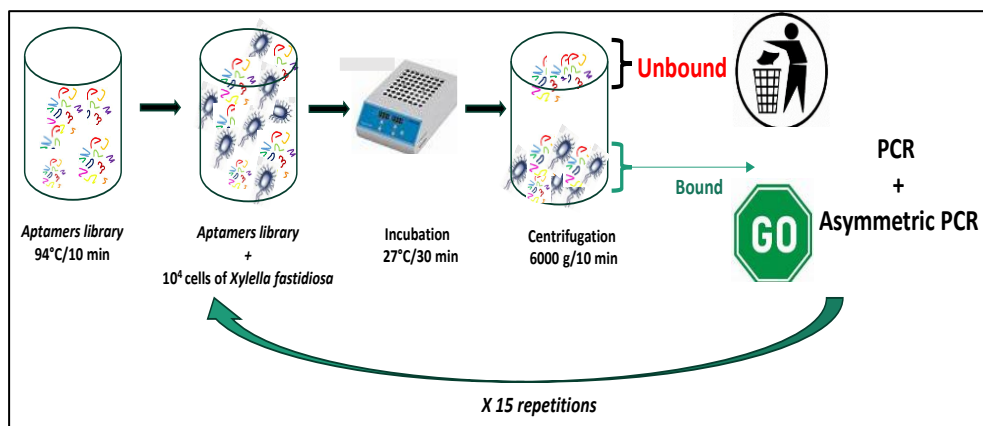
➡ **Bacteriophages (Phages):** highly specific bacterial viruses able to adsorb to, damage and lyse *Xylella* cells for targeted biological control



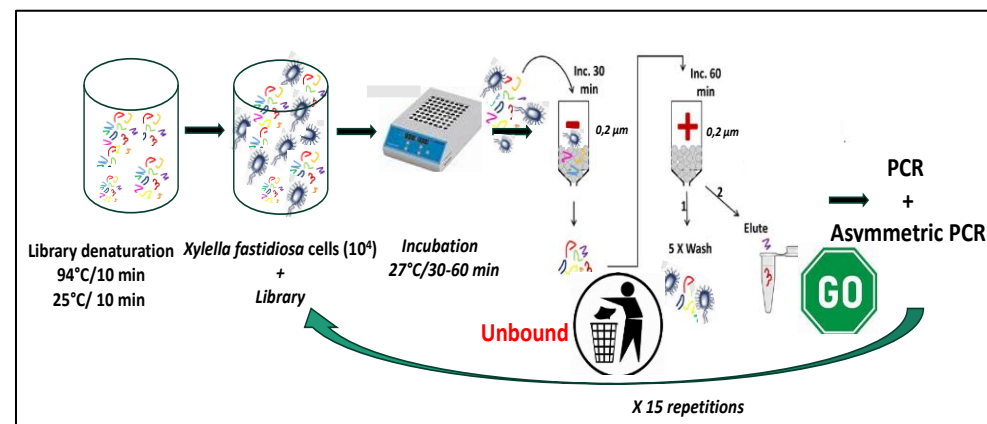
Detection: Aptamer-based Diagnostics

Aptamers Selection Strategy

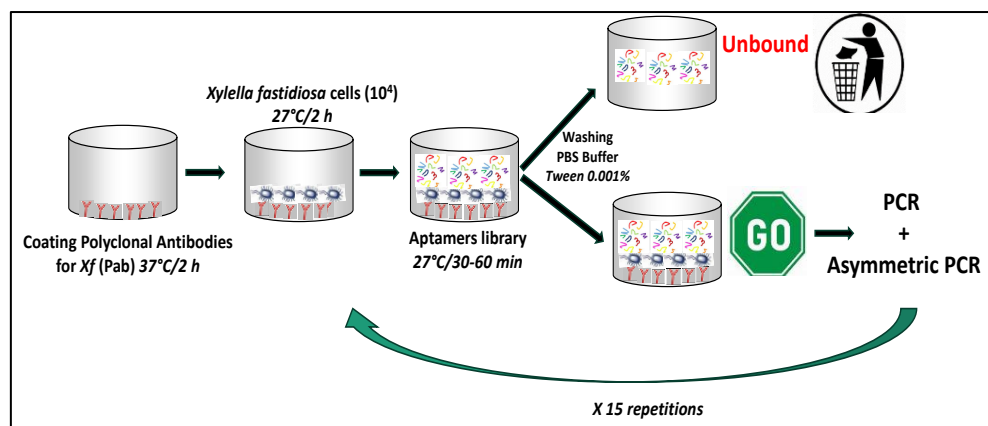
1- Centrifuge-based Cell-SELEX



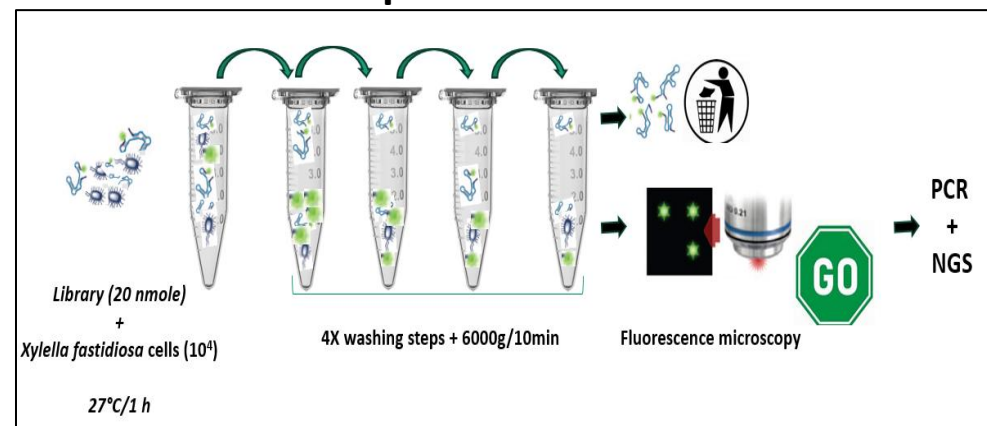
2- Filter-based Cell-SELEX



3- Antibody-based Cell-SELEX



4- One-Step Fluorescence-based Cell



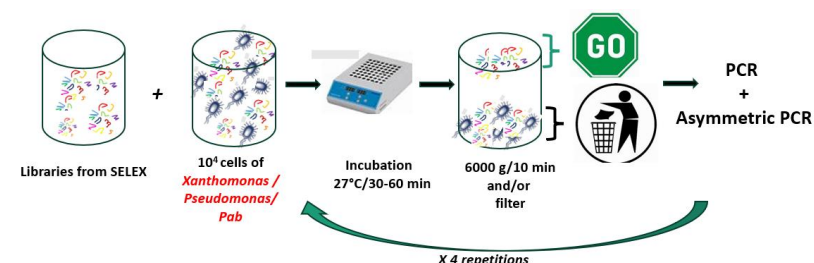
Detection: Aptamer-based Diagnostics

Aptamers Specificity

All Aptamer libraries were subjected to **Counter-Selection** against **non-target bacterial species** (*Xanthomonadaceae*, *Pseudomonadaceae*) to assess and improve their specificity

Epiphytic: *Bacillus safensis*, *Bacillus pumilus*, *Peribacillus frigoritolerans*, *Paenibacillus glucanolyticus*

Endophytic: *Neobacillus niacini*, *Paenibacillus cinensis*, *Bacillus* spp., *Neobacillus* spp



Identification of **22 candidates** from different libraries

Next-Generation Sequencing: DNA Seq of libraries

(1) Centrifuge-based Cell-SELEX (2) Filter-based Cell-SELEX (3) Antibody-based Cell-SELEX

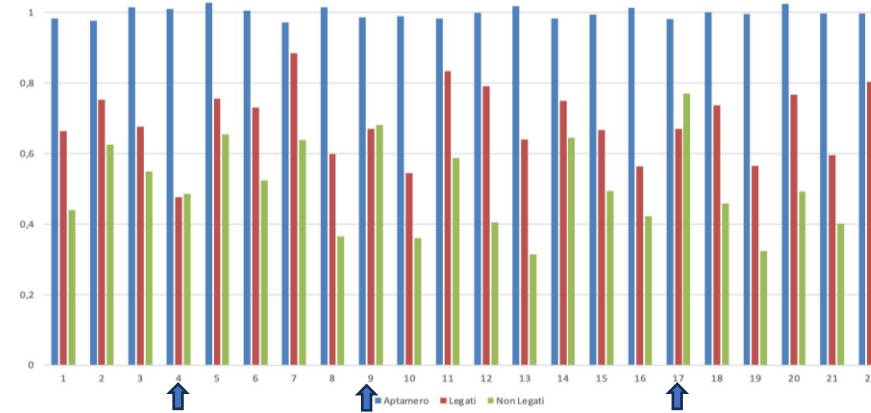
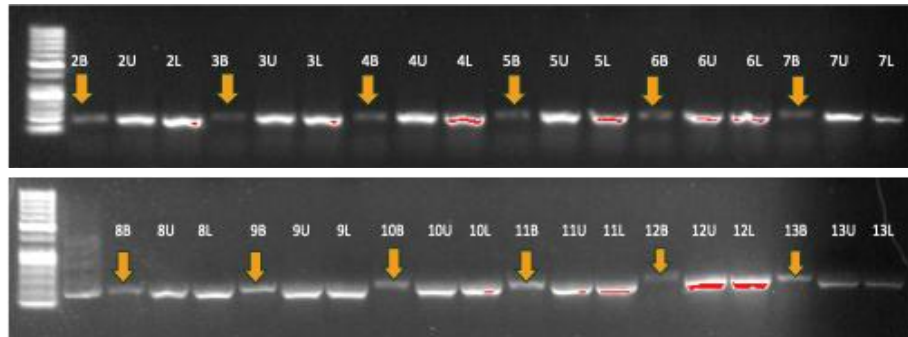
ROUND	READS	ROUND	READS	ROUND	READS
R1	144,148	R1	193,450	R1	199,486
R5	186,328	R5	171,810	R5	187,148
R10	188,756	R10	207,786	R10	147,312
R15	142,644	R15	152,640	R15	138,876
R16 CS(-)	173,416	R16 CS(-)	170,984	R16 CS(-)	135,241



		Arricchimento	
1	GGACAGGACCACACCCAGCG	867	*
2	GGACAGGACCACACCCAGCG	321	*
3	GGACAGGACCACACCCAGCG	107	
4	GGACAGGACCACACCCAGCG	57	
5	GGACAGGACCACACCCAGCG	306	*
6	GGACAGGACCACACCCAGCG	123	*
7	GGACAGGACCACACCCAGCG	141	*
8	GGACAGGACCACACCCAGCG	113	
9	GGACAGGACCACACCCAGCG	60	
10	GGACAGGACCACACCCAGCG	88	
11	GGACAGGACCACACCCAGCG	86	
12	GGACAGGACCACACCCAGCG	78	
13	GGACAGGACCACACCCAGCG	185	*
14	GGACAGGACCACACCCAGCG	225	*
15	GGACAGGACCACACCCAGCG	141	*
16	GGACAGGACCACACCCAGCG	118	
17	GGACAGGACCACACCCAGCG	115	
18	GGACAGGACCACACCCAGCG	84	
19	GGACAGGACCACACCCAGCG	73	
20	GGACAGGACCACACCCAGCG	112	
21	GGACAGGACCACACCCAGCG	47	
22	GGACAGGACCACACCCAGCG		

Detection: Aptamer-based Diagnostics

Evaluation of Single Aptamer Binding Specificity to Xylella by PCR\qPCR



10 Best performing Aptamers are selected

- High amplification efficiency
- Strong enrichment in previous NGS analyses



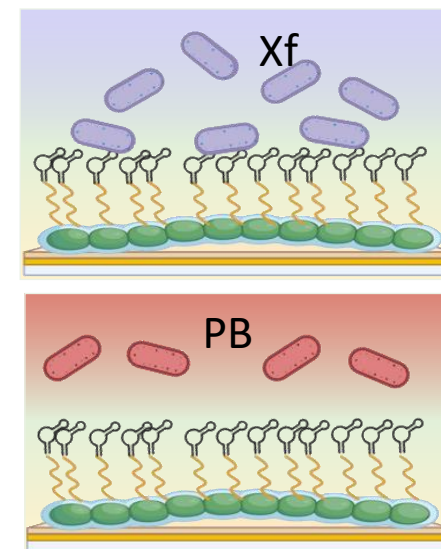
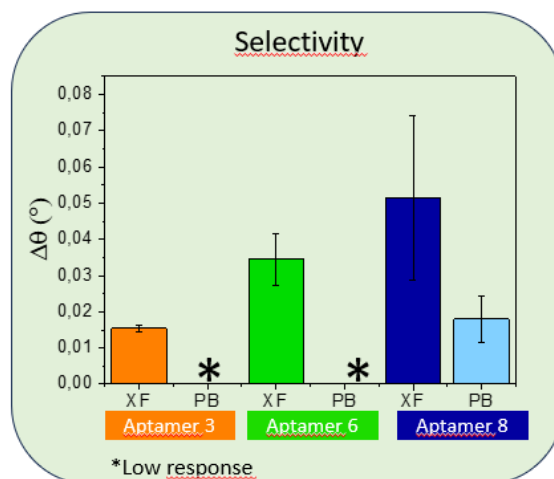
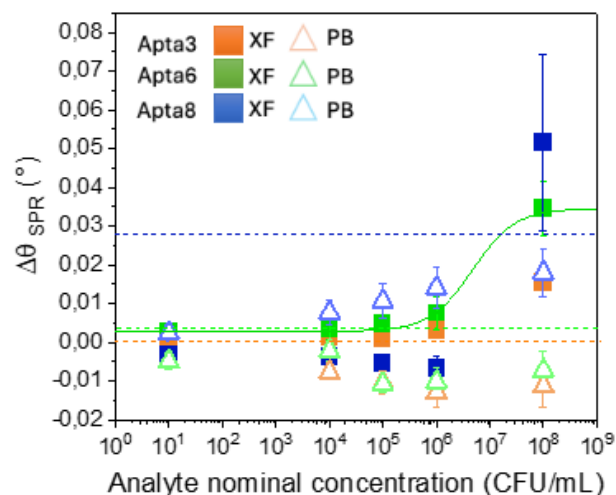
Surface Plasmon Resonance (SPR)



Detection: Aptamer-based Diagnostics

SPR Results: Evaluating aptamer binding to *Xylella* and compare the response against non-target bacteria

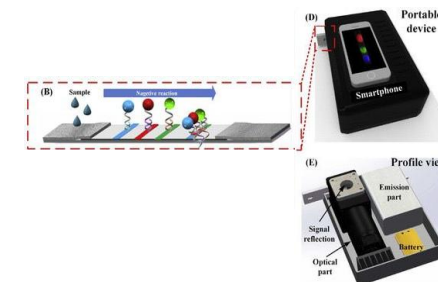
3 out of 10 Aptamers showed specific recognition of *Xylella* and support the development of portable diagnostic devices (Smart, Rapid, Cost-Effective, Innovative Tool (SiMoT))



PB: *Paraburkholderia phytofirmans*

Equation:	$y = \frac{Vmax \times x^n}{k^n + x^n}$
k	$(5 \pm 3) \cdot 10^6$
n	$1,1 \pm 0,5$
$K^n = K_D$	$2,3 \cdot 10^7$ CFU/mL

Association rate, k_a ; Dissociation rate, k_d ; Binding affinity, K_D



Control strategy

From in vitro activity to field-applicable biological control



BACTERIOCIN

Nisin A

Lactococcus lactis



BACTERIOCIN

MS4 bacteriocins

Leuconostoc mesenteroides



BACTERIOPHAGE

Selected phages

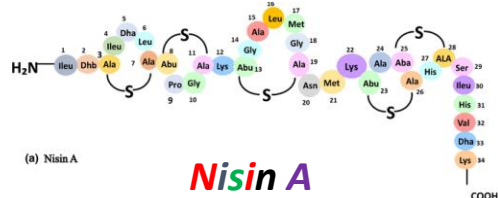
Targeted Xylella lysis

In vitro activity



Field-applicable biological control

Antimicrobial peptide strategy: *Lactococcus lactis* / Nisin A



Nisin A activity against *Xylella*

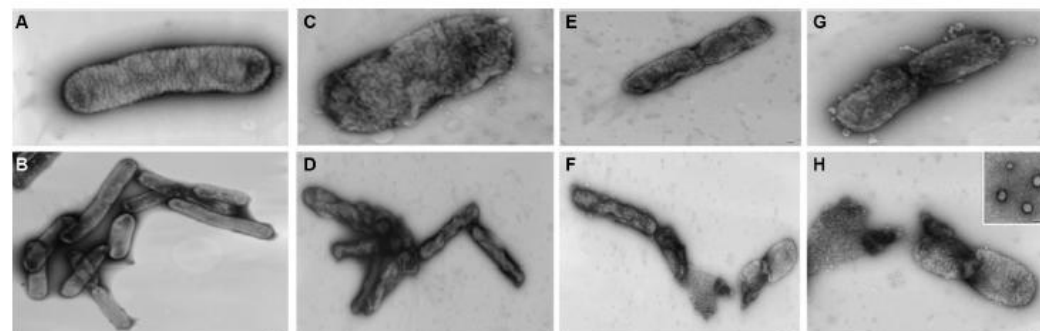
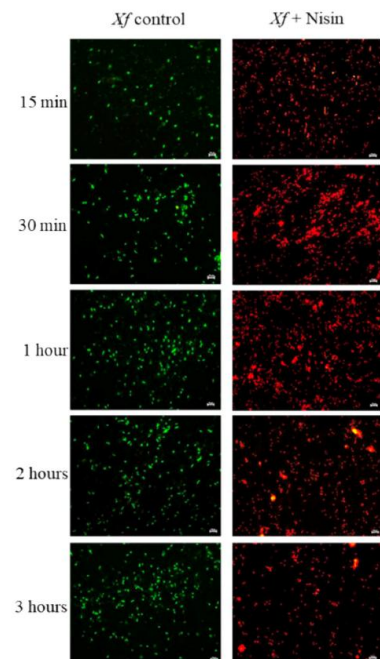
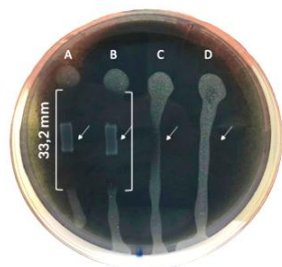
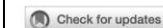


FIGURE 8
Transmission electron micrographs showing the impact of nisin on *Xf* cells. (A,B) Untreated *Xf* cells, used as control. (C–H) Nisin-treated *Xf* cells showing structural, cell wall, and cytoplasm alterations. Bar: 100 nm.



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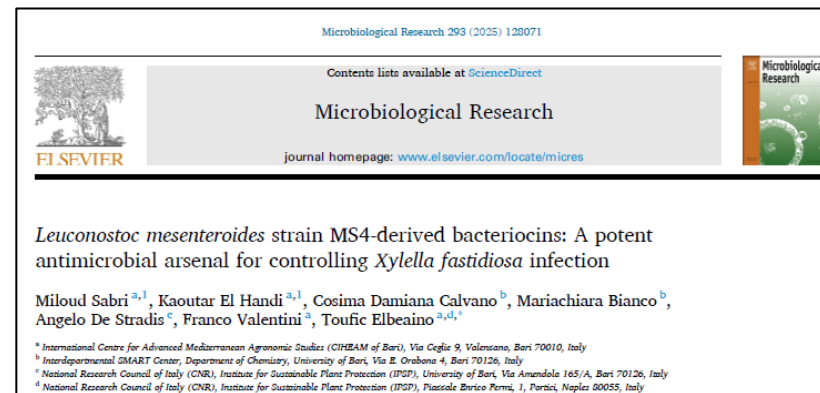
Nisin-based therapy: a realistic and eco-friendly biocontrol strategy to contrast *Xylella fastidiosa* subsp. *pauca* infections in planta

Miloud Sabri^{1†}, Kaoutar El Handi^{1†}, Franco Valentini¹,
Angelo De Stradis², Orges Cara^{1,3}, Cosima Damiana Calvano⁴,
Mariachiara Bianco⁴, Antonio Trani¹ and Toufic Elbeaino^{1,5*}

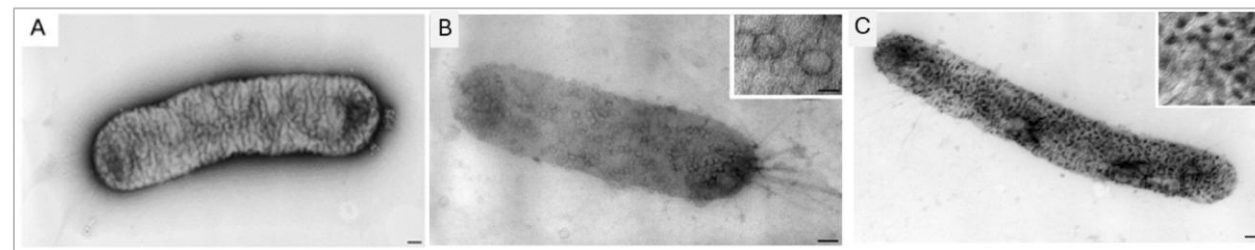
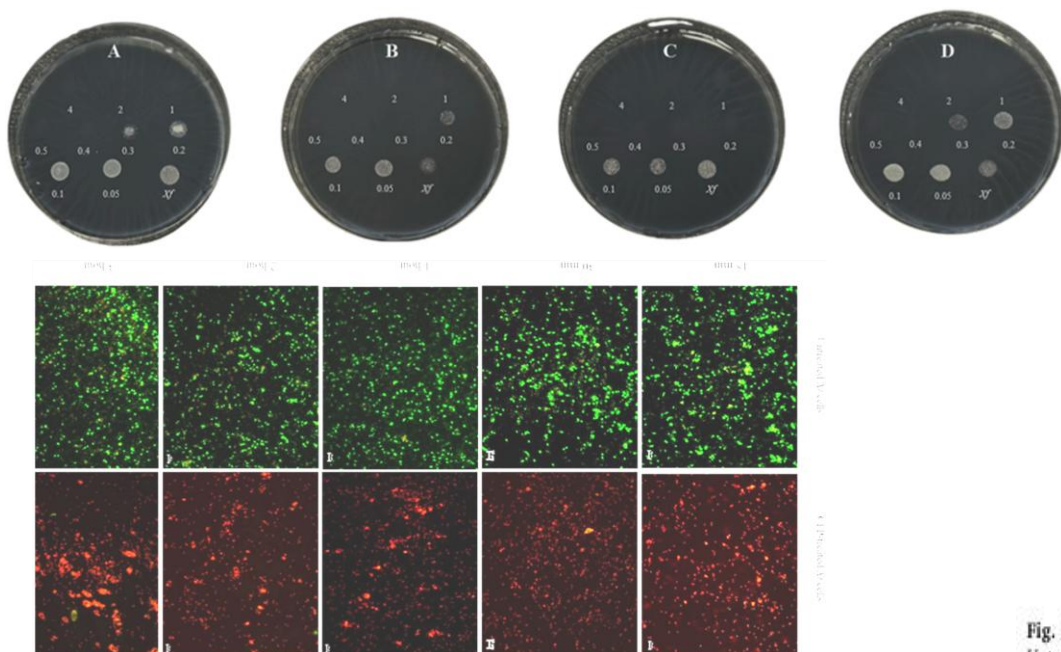


Antimicrobial peptide strategies: *Leuconostoc mesenteroides* MS4

Sequence	Amino acids length	Laboratory code	NCBI similarity (closest match & accession)	Identity (%)
MTLNKKLQNSLHGYDILDAQQLEVDGGIAPLLIAGGIVLAGGGFAAGYGLSRWL	58	MG-58	class IIb bacteriocin, lactobin A/cerein 7B family (WP_010287275.1)	100 %
MIKNDLNFNSQLEDEYQLASVDGGFIPVAVWALWAGAGAAGSGGIAGVGLNRNRNN	59	MN-59	class IIb bacteriocin, lactobin A/cerein 7B family (WP_010287277.1)	100 %
MSLKNIVGFNKLDSLEKNINGGCPYLLIGALAGAIRNRIKK	45	MK-45	class IIb bacteriocin, lactobin A/cerein 7B family (WP_071951754.1)	100 %
MALFKLSNKLNVNNGGVVPVAVVLGVPITLALGIYQAGYAAKGDRAGAGLR	53	MR-53	class IIb bacteriocin, lactobin A/cerein 7B family (WP_010287029.1)	100 %
MINSTAVKELNDMELESINGGWSPOGFAISFVLGGAVGVGAYSIFYQGMDDQGYRNNKK	58	MK-58	class IIb bacteriocin, lactobin A/cerein 7B family (WP_010287032.1)	100 %
MEKLSQELAKVSGGFPPLPVPPIAGGATYAKDAWNHLQIRSGWRKAGNSKW	56	MW-56	bacteriocin (WP_010288466.1)	100 %



4 out of 6 bacteriocins showed anti-*Xylella* activity



Effect of MS4 cell-free precipitate on *Xfp*

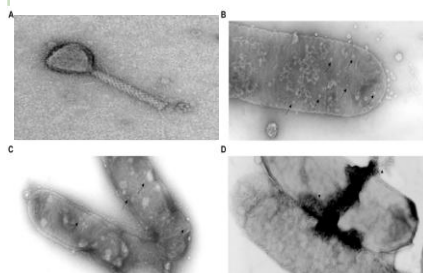


Fig. 9. In planta assays showing the effect of MS4 cell-free precipitate on *Xylella fastidiosa* subsp. *pauca* in *Nicotiana benthamiana* plants, 74 dpi. A. Untreated *Xfp*-infected plants showing leaf scorch symptoms (indicated by arrows). B. Healthy plants showing no symptoms. C. Preventive CFP-treated *Xfp*-infected plants with no symptoms. D. Curative CFP-treated *Xfp*-infected plants with no symptoms.

Newly discovered Phages active against *Xylella fastidiosa*

Phage MATE2

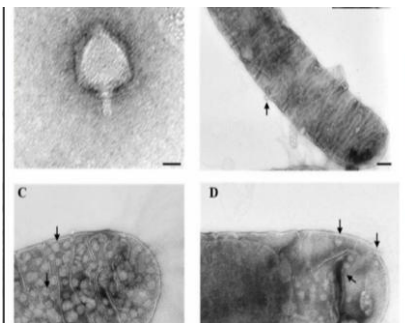
Carpasnavirus
(63.695 bp)



TEM: Lytic activity against Xfp

Phage Phi1

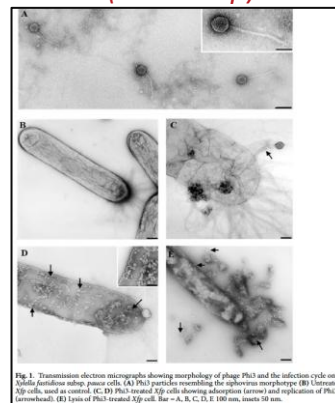
Pradovirus
(44.345 bp)



TEM: Interaction with Xf cells and observed cellular changes

Phage Phi 3

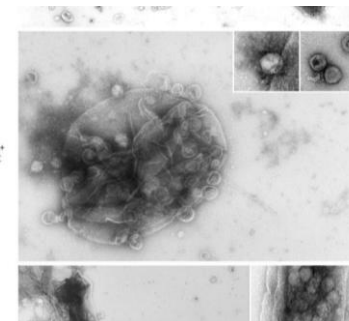
Salvovirus
(55.413 bp)



TEM: adsorption, cellular damage, and lysis

Phage PhiBC

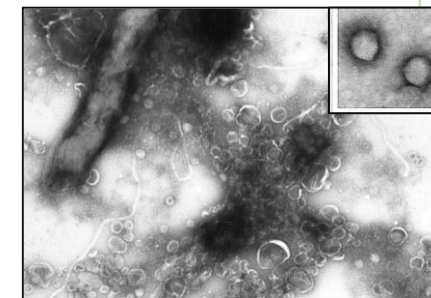
Classification pending
(45.048 bp)



TEM: adsorption, cellular damage, and lysis

Phage PhiD

Classification pending
(44.484 bp)



TEM: Interaction/aggregation with Xf cells

frontiers | Frontiers in Microbiology

TYPE: Original Research
PUBLISHED: 28 May 2024
DOI: 10.3389/fmicb.2024.1412650

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Xylella phage MATE 2: a novel bacteriophage with potent lytic activity against *Xylella fastidiosa* subsp. *pauca*

Miloud Sabri^{1†}, Kaoutar El Handi^{1†}, Orges Cara^{1,2}, Angelo De Stradis³, Franco Valentini¹ and Toufic Elbeaino^{1,4*}

¹International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM of Bari), Valenzano, Italy; ²Department of Soil, Plant and Food Science, University of Bari, Bari, Italy; ³Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (CNR), University of Bari, Bari, Italy; ⁴Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (CNR), Portici, Italy

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Identification and characterization of Phi1 and Phi3 bacteriophages targeting *Xylella fastidiosa* subsp. *pauca*, the causal agent of olive quick decline in Italy

Miloud Sabri, Kaoutar El Handi, Khaoula Mektoubi, Orges Cara, Angelo De Stradis, Franco Valentini & Toufic Elbeaino

[Scientific Reports](#) 16, Article number: 11969 (2026) | [Cite this article](#)

2026

Ongoing screenhouses and field experimental trials against *Xylella fastidiosa*

- **Treatments:** *Nisin*, *L. mesenteroides*/bacteriocins, and a mixture of selected phages
- **Application method:** Trunk injection and/or soil drenching
- **Plant material:** *Ogliarola* and *Leccino* cultivars, subject to experimental availability
- **Biological and Molecular Assessments:** symptoms, plant survival, and reduction in the bacterial load of *Xylella*

Application: trunk injection



Application: soil drenching



Field experimental trials

Francavilla Fontana, Brindisi province

40°29'56.0"N 17°34'13.7"E



Funding and Acknowledgements



“Ottimizzazione delle tecniche di diagnosi della presenza di Xylella fastidiosa su matrici vegetali e su insetti vettori: controllo e diagnostica

*(Progetto **1LIVEXYLELLA**, CUP H93C22000760006)*



“Approcci Nanotecnologici per un Controllo Sostenibile e Innovativo di Xylella fastidiosa
*(Progetto **ANCOSIX**, CUP n. J83C22001990005)*

Team of Researchers and Partner Institutions



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(Dept of IPM)



Toufic Elbeaino



Miloud Sabri



Orges Cara



Amani Ben Slimen



Franco Valentini



UNIBA
(Dept of Chemistry)



Luisa Torsi



Eleonora Macchia



Lucia Sarcina



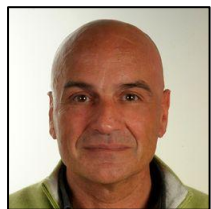
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THANK YOU

Questions & discussion

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

Pau Caravaca-Fuentes^{1,2}, Laura Montesinos¹, Jordi Lamata², Marta Planas², Lidia Feliu², Emilio Montesinos¹

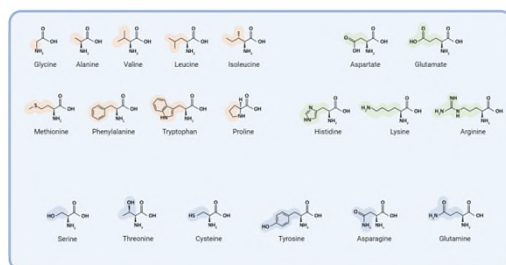
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²LIPPSO, Chemistry Department and Institute of Agrifood Technology, University of Girona, Girona, Spain

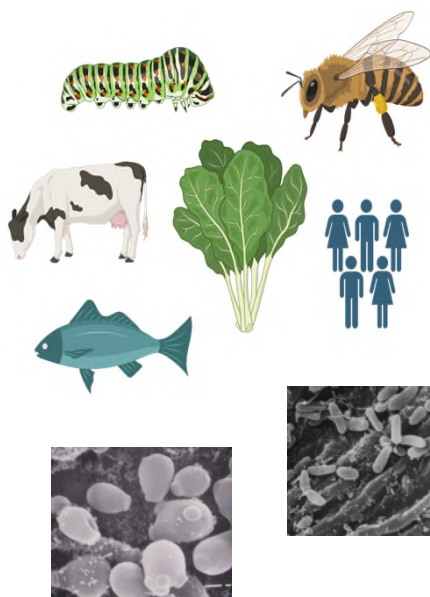


From natural peptides to synthetic analogs that target *Xylella fastidiosa* or the host plant

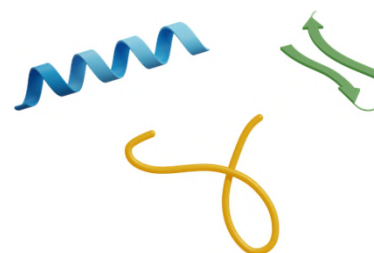
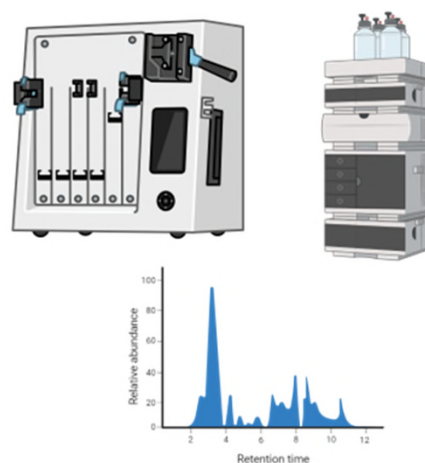
Sources of natural peptides



Glu His Phe Arg Trp Gly Lys Pro Val Gly Lys Lys Arg Arg Pro Val Lys Val Tyr Pro

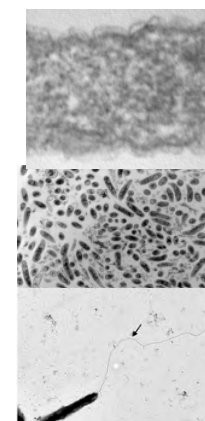


Synthesis of analogs Peptide libraries



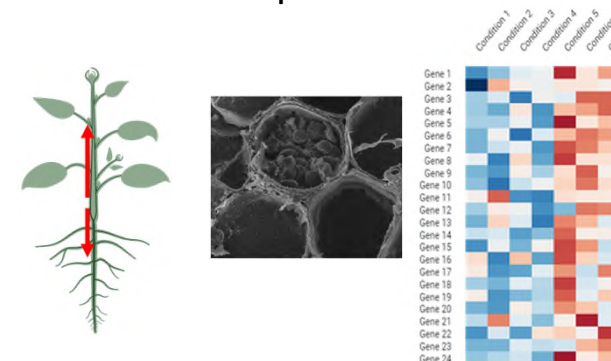
Screening activities

Pathogen cell targets



Membranes
Biofilm
Motility
RNA interference
...

Induction of plant host defence



Functional peptides active against *Xylella fastidiosa*

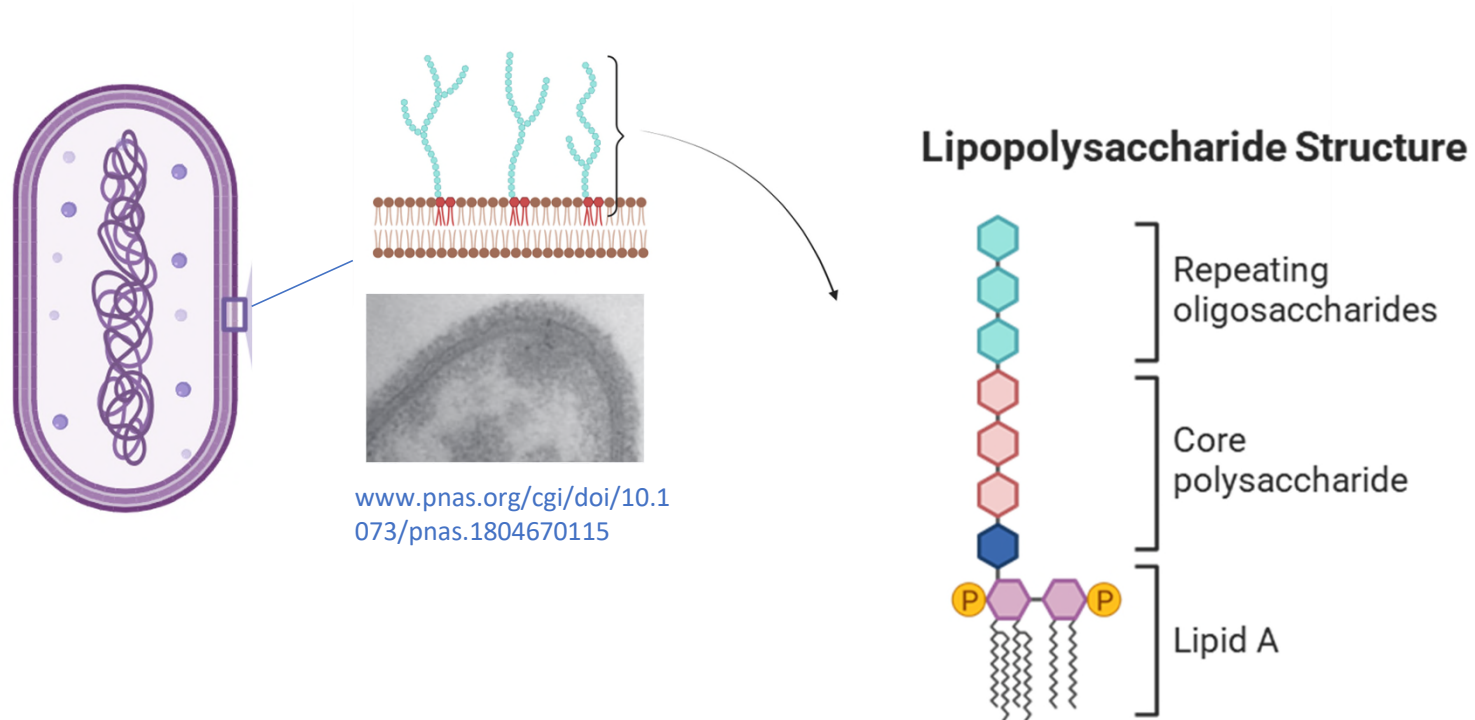
Peptide	Sequence	Bac	Ab	Mt	Df	References
Indolicidin	ILPWKWPWWPWRR-NH ₂	✓	✓	-	-	Kuzina et al., 2006; Moll et al., 2021
Magainin 2	GIGKFLHSAKKFGKAFVGEIMNS-NH ₂	X	✓	-	-	Kuzina et al., 2006; Moll et al., 2021
BP171	KKLFKKILKYLGPATTGLPALISW-OH	✓	-	-	-	Baró et al., 2020a, 2020b
BP178	KKLFKKILKYLGPAGIGKFLHSAKKDEL-OH	✓	-	-	✓	Baró et al., 2020a, 2020b; Moll et al., 2022
1026	VQWRIRVRVIKK-NH ₂	✓	✓	-	-	De La Fuente-Núñez et al., 2012; Moll et al., 2021
1036	VQFRIRVRIVIRK-NH ₂	✓	✓	X	✓	De La Fuente-Núñez et al., 2012; Moll et al., 2021, 2025
RIJK2	RIVWVRIRRWV-NH ₂	✓	✓	X	✓	De La Fuente-Núñez et al., 2015; Moll et al., 2021, 2024, 2025
RR4-OH	WLRRIKAWLRRIKA-OH	X	✓	-	✓	Mohamed et al., 2017; Moll et al., 2021, 2024
FV7	FRIRVRV-NH ₂	X	✓	X	✓	Moll et al., 2021, 2024, 2025; Xu et al., 2014
R-FV7-I16	RFRRLLFRIRVRVLKKI-NH ₂	X	✓	X	-	Moll et al., 2021, 2024, 2025; Xu et al. 2014
BP100	KKLFKKILKYL-NH ₂	X	X	✓	✓	Moll et al., 2021, 2024, 2025
BP16	KKLFKKILKKL-NH ₂	X	X	X	✓	Moll et al., 2021, 2024, 2025
KSLW-FV7	KKVVFVVKFKFRIRVRV-NH ₂	✓	✓	X	✓	Moll et al., 2024, 2025
FV7-KSLW	FRIRVRVKVVFVVKFK-NH ₂	X	✓	✓	-	Moll et al., 2025
flg22-NH ₂	QRLSTGSRINSKDDAAGLQIA-NH ₂	X	X	-	✓	Moll et al., 2024

Bac – bactericidal; Ab –antibiofilm; Mt – antimotility; Df – induction of defences

✓ yes; X No; - not determined

Several synthetic peptides have been described as active against *X. fastidiosa*

The lipopolysaccharides (LPS) of *X. fastidiosa* as a target for functional peptides



LPSs are critical virulence factors anchored to the bacterial membrane.

- Immune evasion
- Biofilm formation
- Membrane integrity and permeability



ARTICLE

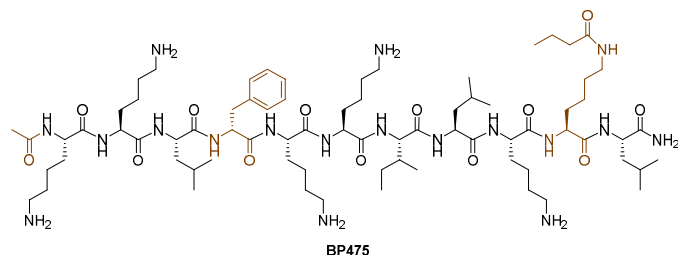
DOI: 10.1038/s41467-018-02841-0

OPEN

Lipopolysaccharide O-antigen delays plant innate immune recognition of *Xylella fastidiosa*

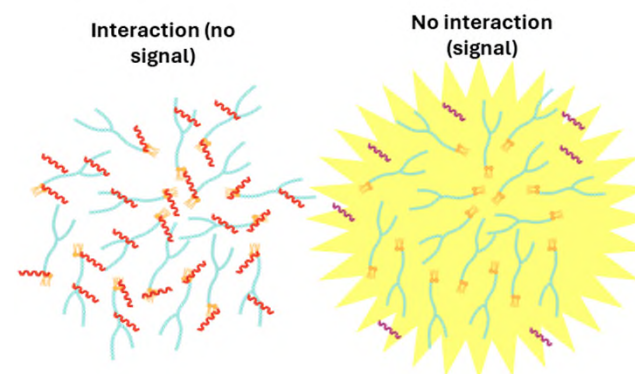
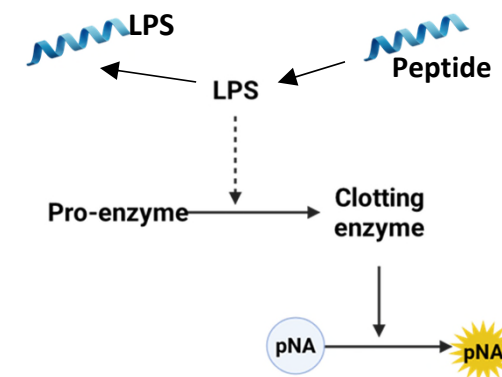
Jeannette N. Rapicavoli¹, Barbara Blanco-Ulate^{2,3}, Artur Muszyński⁴, Rosa Figueroa-Balderas², Abraham Morales-Cruz², Parastoo Azadi⁴, Justyna M. Dobruchowska⁴, Claudia Castro¹, Dario Cantu² & M. Caroline Roper¹

Lipopeptides derived from BP100 and interaction with LPS

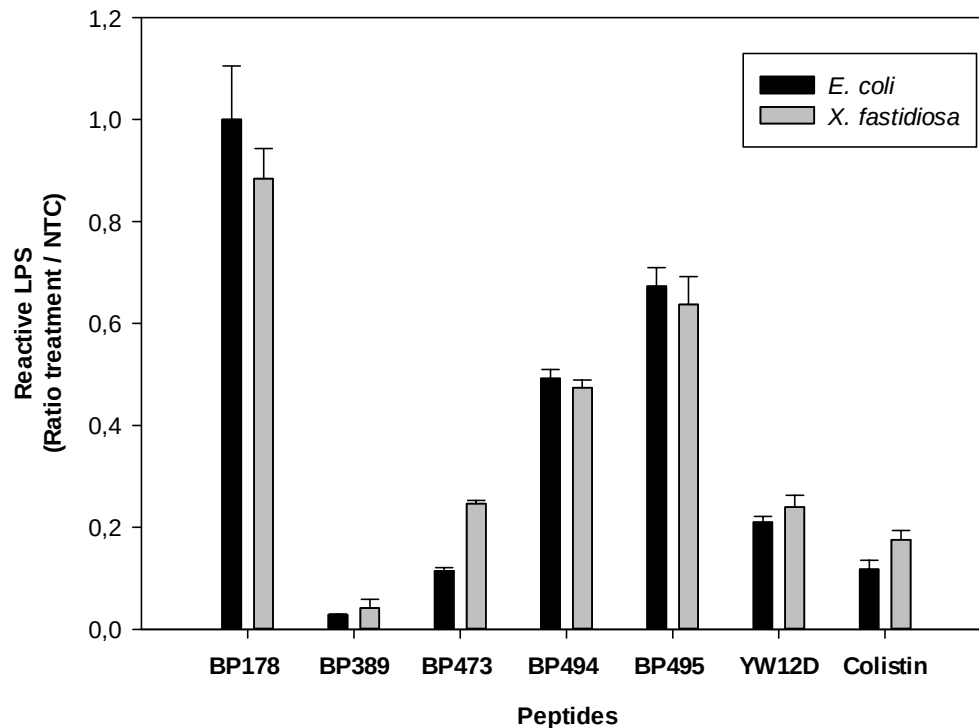


Code	Sequence
BP375	Ac-KKLFKKIK(COC ₅ H ₁₁)KYL-NH ₂
BP377	Ac-KKLFKKILKK(COC ₅ H ₁₁)L-NH ₂
BP387	Ac-KKLFKKIK(COC ₃ H ₇)KYL-NH ₂
BP389	Ac-KKLFKKILKK(COC ₃ H ₇)L-NH ₂
BP393	Ac-KK(COC ₁₁ H ₂₃)LFKKILKYL-NH ₂
BP473	Ac-KKLfKK(COC ₃ H ₇)ILKYL-NH ₂
BP474	Ac-KKLfKKIK(COC ₃ H ₇)KYL-NH ₂
BP475	Ac-KKLfKKILKK(COC ₃ H ₇)L-NH ₂
BP485	C ₃ H ₇ CO-KKLfKKILKYL-NH ₂
BP490	Ac-KKLfKKIK(COC ₁₁ H ₂₃)KYL-NH ₂
BP494	Ac-KKLfKKK(COC ₅ H ₁₁)LKYL-NH ₂
BP495	Ac-KKLfKKILKYK(COC ₅ H ₁₁)-NH ₂
BP496	Ac-KKLfKKILKYK(COC ₃ H ₇)-NH ₂
BP498	Ac-KKLfK(COC ₃ H ₇)KILKYL-NH ₂
BP499	Ac-KKLfKKILK(COC ₃ H ₇)YL-NH ₂
BP500	Ac-KKK(COC ₁₁ H ₂₃)fKKILKYL-NH ₂
BP501	Ac-KKLHKILKK(COC ₃ H ₇)L-NH ₂
BP545	Ac-K(COC ₃ H ₇)KLfKKILKYL-NH ₂
BP546	Ac-KKK(COC ₃ H ₇)fKKKLKYL-NH ₂
BP547	Ac-KKLk(COC ₃ H ₇)KKILIYL-NH ₂
BP548	Ac-KKLfKKK(COC ₃ H ₇)LKYL-NH ₂
BP549	Ac-KKK(COC ₃ H ₇)fKKILKYL-NH ₂
BP550	Ac-KKLk(COC ₃ H ₇)KKILKYL-NH ₂

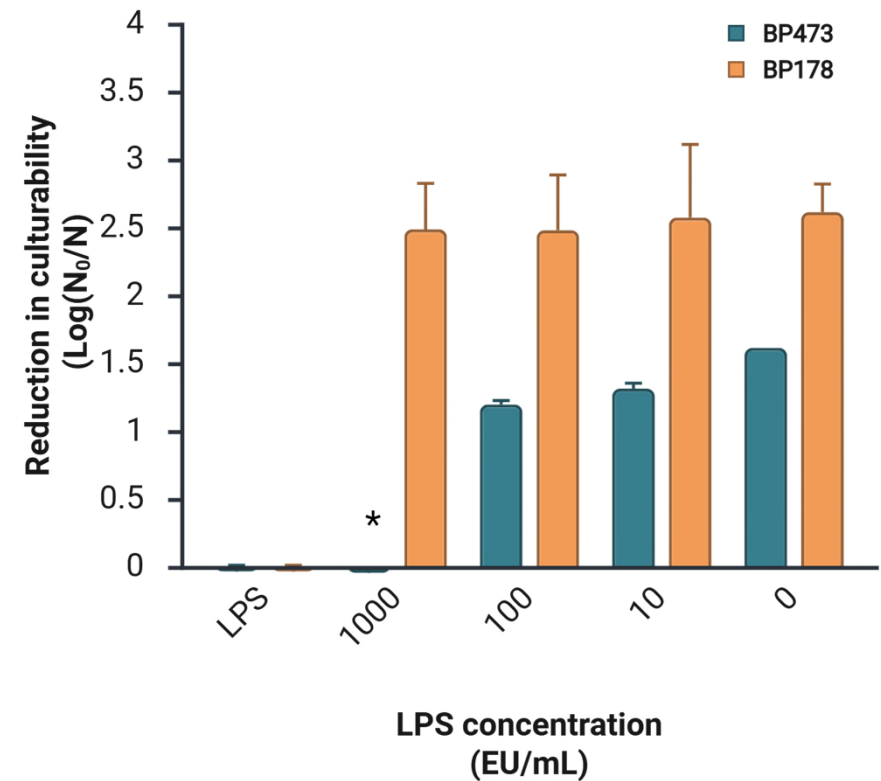
Limulus Lysate Test for endotoxins (LAL) Peptide-LPS interaction



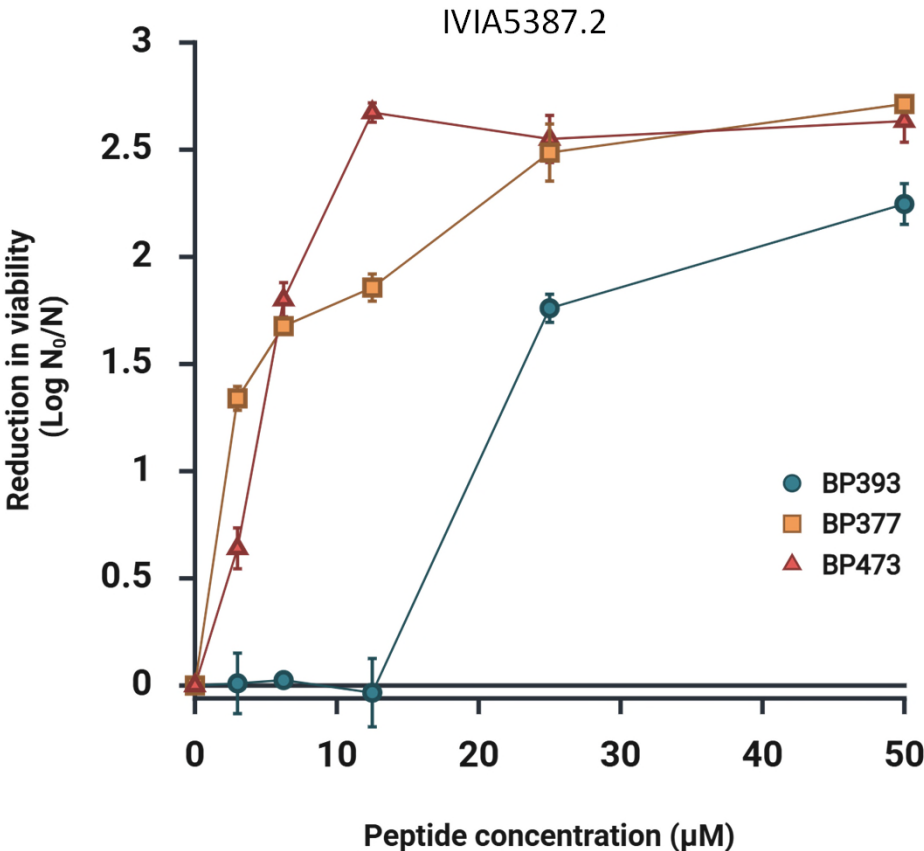
LPS neutralization



Quenching of antibacterial peptide activity by LPS



Several lead peptides strongly interact with LPSs



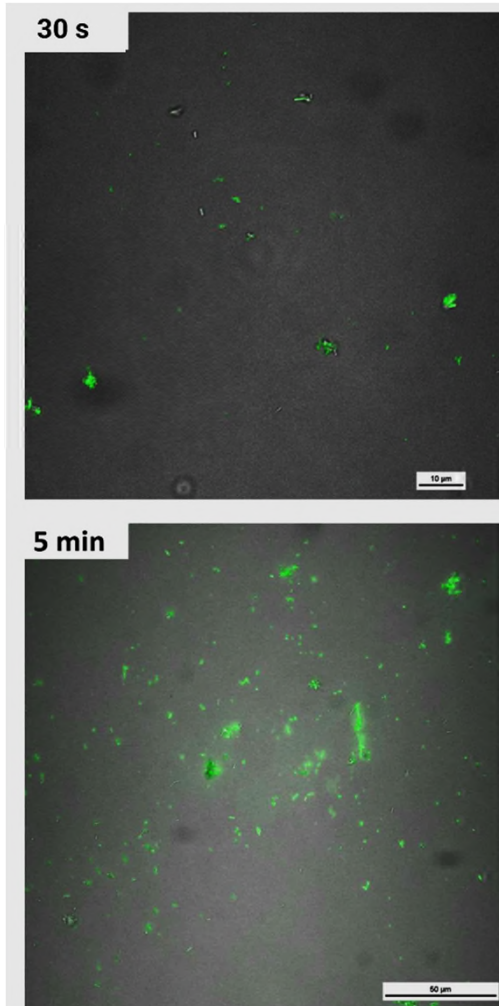
		BP473		BP178		BP100	
		25 μM	12.5 μM	25 μM	12.5 μM	25 μM	12.5 μM
fastidiosa	Temecula	2.8	2.9	2.4	1.9	2.4	2.0
	IVIA 5387.2	2.6	2.7	2.6	2.0	1.6	1.6
	IVIA 5770	3.3	2.4	3.0	2.5	2.8	2.7
multiplex	IVIA 5901.2	2.3	2.3	2.2	2.3	2.3	2.1
	CFBP1873	3.0	2.6	3.1	2.1	2.9	2.1
pauca	CN-28	0.52	0.19	0.72	0.48	0.30	0
	DeDonno	0.76	0.39	1.4	0.88	0.52	0.26

Reduction in viability Log(N_0/N)

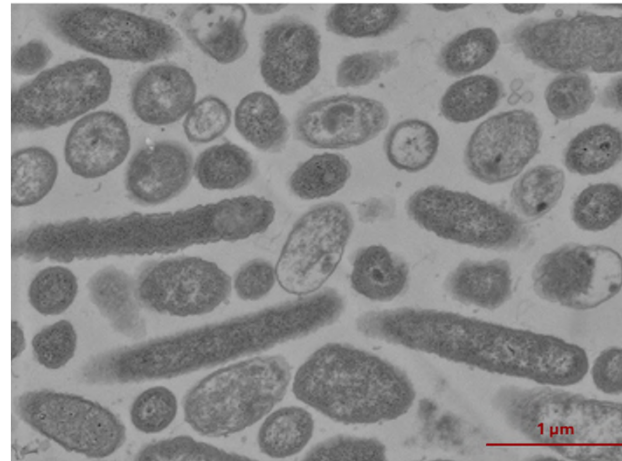
The best peptide interacting with LPSs is also bactericidal against strains of *X. fastidiosa* but at different level

Cell interaction and lysis of *X. fastidiosa* by peptide BP473

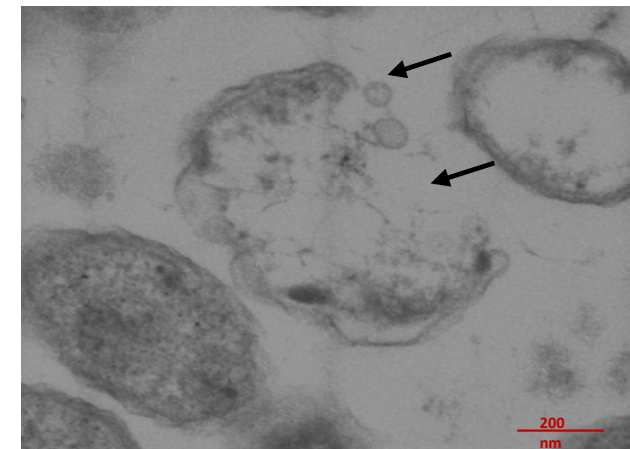
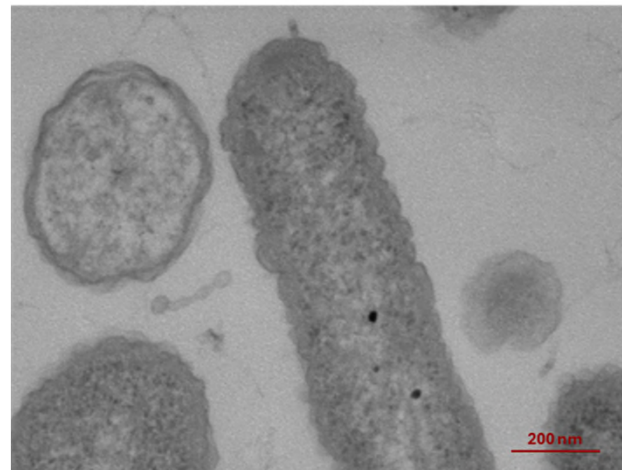
BP473-K(CF)



NTC



BP473



Peptide BP473 interacts with *X. fastidiosa* cells and produce cell lysis

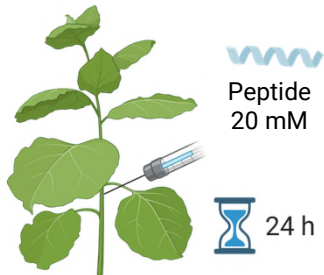
Nicotiana benthamiana as a plant model for *X. fastidiosa*

Advantages

- Rapid growth
- Easy transformation
- Susceptibility to pathogens (*X. fastidiosa*)
- Endotherapy in adult plants



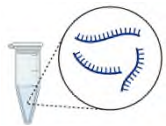
1 Treatment by endotherapy



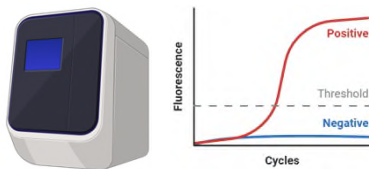
2 Samples



3 RNA extraction



4 RT-qPCR



Fold change ($2^{-\Delta\Delta C_t}$) normalized to actin

FC > 1.5 overexpression
FC < 0.5 repression

Genes	BP178	BP473	Pathway
<i>ERF1</i>	1.58	1.48	JA/ET
<i>9LOX</i>	2.24	2.93	Oxylipins (Indirect-Ja)
<i>PR3</i>	4.07	0.74	JA/ET
<i>PAL</i>	1.40	1.39	Phenylpropanoids (Indirect-SA)
<i>PAL2</i>	0.76	0.90	Phenylpropanoids (Indirect-SA)
<i>PR1</i>	23.78	1.27	SA
<i>PR2</i>	7.20	1.36	SA
<i>PR4a</i>	0.82	0.43	JA/ET
<i>WRKY25</i>	1.05	1.31	TF (cross-talk SA-JA-ET)
<i>MAPK3</i>	1.63	2.08	PTI/MAPK (early signaling)
<i>PR4</i>	0.63	0.43	JA/ET

SA: salicylic acid

JA: jasmonic acid

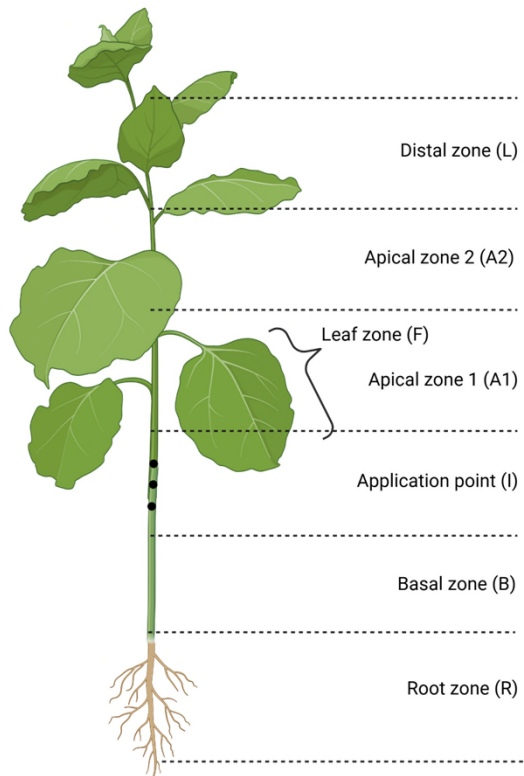
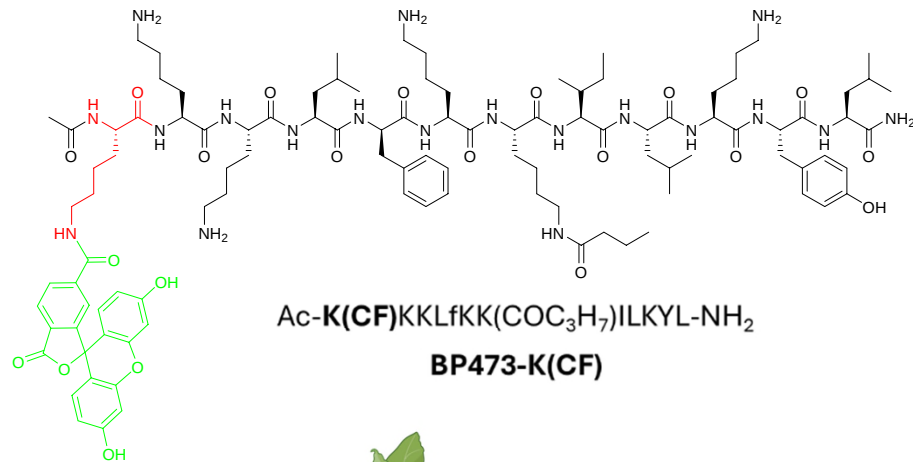
ET: ethylene

TF: transcription factor

PTI: Pattern-triggered immunity

Peptide BP473 do not induce a significant plant response at transcriptomic level

Systemic translocation of peptide BP473



Dia 3 / Zona I / BP473-K(CF)



Dia 0 / Zona I (Pela) / BP473-K(CF)



Dia 3 / Zona F / BP473-K(CF)



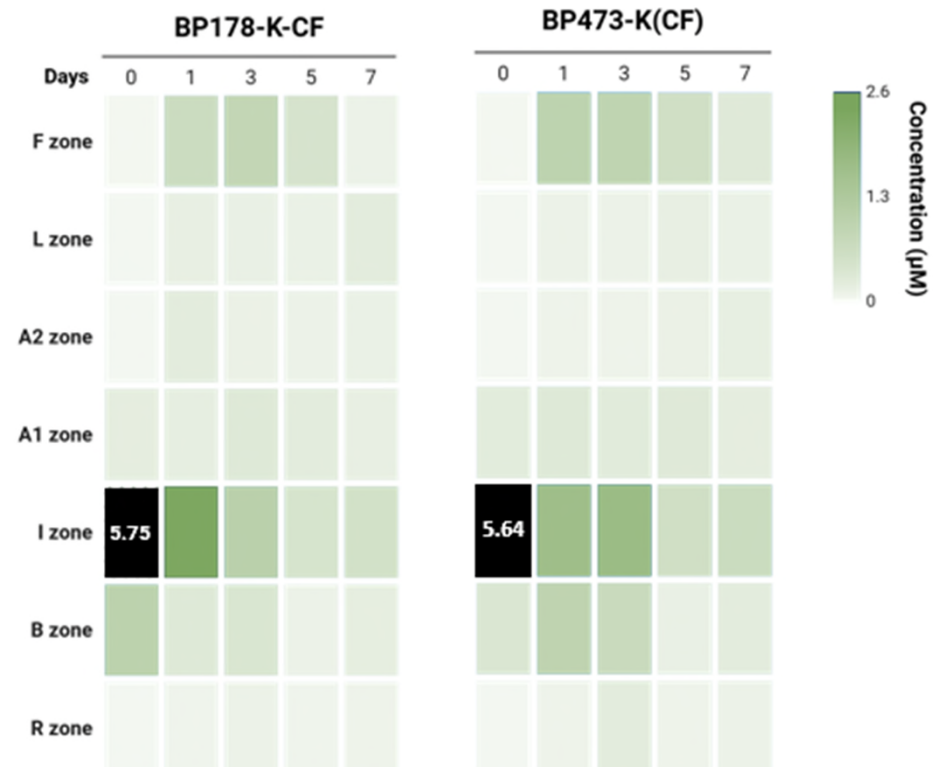
Dia 0 / Zona I / BP178-K-CF



Dia 3 / Zona I / BP178-K-CF



Dia 7 / Zona I / BP178-K-CF

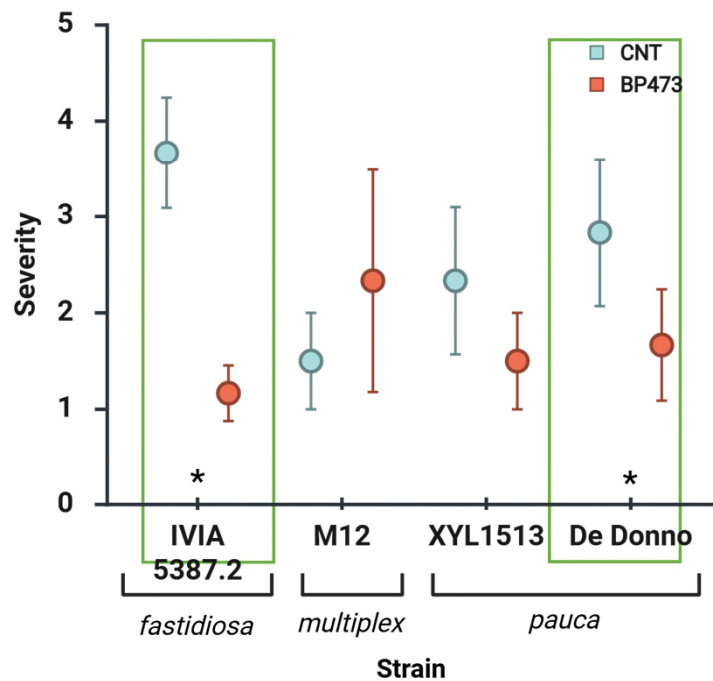


Peptide BP473 is translocated through the vascular system of the plant mainly upwards

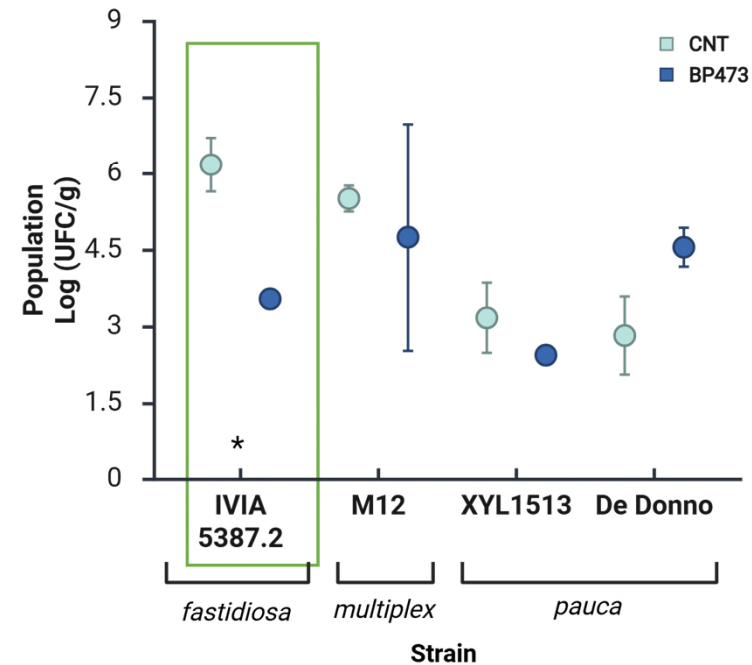
Infection control assays. 65 dpi.



Symptoms



Population level



Peptide BP473 applied by endotherapy decreases population levels of *X. fastidiosa* and symptoms, but only in some strains



BEXYL
BEYOND XYLELLA



TED2021-130110B-C43
PID2022-140040OB-C21
101060593
FPU20/07152



EVALUATION OF THERMOTHERAPY PROTOCOLS ON NURSERY PLANT MATERIAL FOR THE MANAGEMENT OF *XYLELLA FASTIDIOSA* RISK

M. Casetta, E. Dongiovanni, P. Venerito, S. Amoia, M. Saponari, P. La Notte



Centro di Ricerca
Sperimentazione e Formazione
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Consiglio Nazionale delle Ricerche
Istituto per la Protezione Sostenibile delle Piante



Consiglio Nazionale delle Ricerche
Istituto per la Protezione Sostenibile delle Piante



REGIONE PUGLIA

INTRODUCTION

➤ *Xylella fastidiosa*:

- Quarantine pathogen in the EU
- More than 600 host species
- Causes severe diseases:
 - Olive Quick Decline Syndrome
 - Pierce's Disease of grapevine
 - Leaf scorch of fig and mulberry

➤ Pathways of spread:

- Infected plant → Vectors → Local spread (field to field)
- Infected nursery stock → Trade & Movement → Long-distance spread (region to region or region to country)

➤ Effective sanitation treatments are essential to:

- Prevent pathogen spread
- Ensure safe plant propagation



Olive Quick Decline Syndrome



Pierce's Disease of grapevine

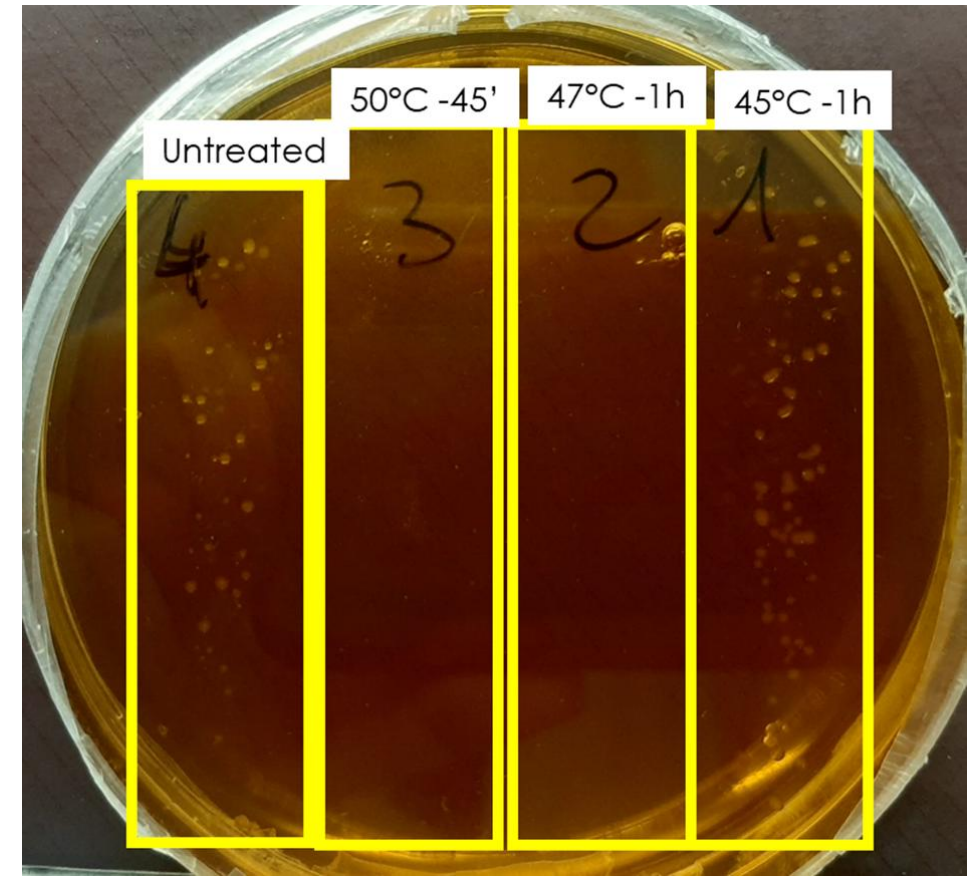
➤ **BeXyl Project:**

- A dedicated work package investigated the use of thermotherapy as a potential strategy to control the bacterium in propagation material

➤ **Why thermotherapy?**

- Thermotherapy is a well-established approach in plant health management;
- The HWT protocol at 50°C-45 min is recognized by EFSA for grapevine;
- Preliminary laboratory studies within the BeXyl project showed that the growth of *Xylella fastidiosa* can be inhibited by high temperatures, based on thermotherapy treatments applied to fresh cuttings from infected grapevines.

THE OBJECTIVE OF THIS WORK WAS TO IDENTIFY THERMOTHERAPY CONDITIONS ABLE TO EFFECTIVELY CONTROL *X. FASTIDIOSA* WHILE PRESERVING THE HEALTH AND VIABILITY OF THE TREATED PLANT MATERIAL



MATERIAL AND METHODS

➤ Experimental Trials:

- **Period:** 2024-2026
- **Host species:** different deciduous or evergreen host species of *X. fastidiosa*
- **Propagation material:** three types of propagation material:
 - ☐ Bare-root plants
 - ☐ Cuttings
 - ☐ Scions for grafting

➤ Hot Water Treatment (HWT) process:

Before thermal treatment

Collection and cold storage of propagation material (4°C)

Acclimation of plant material at room-temperature for 12-24 h

Thermal treatment

HWT of plant material under different time-Temperature combinations

45° C – 50 min
48°C – 45 min
50°C – 45 min

Immediately after thermal treatment

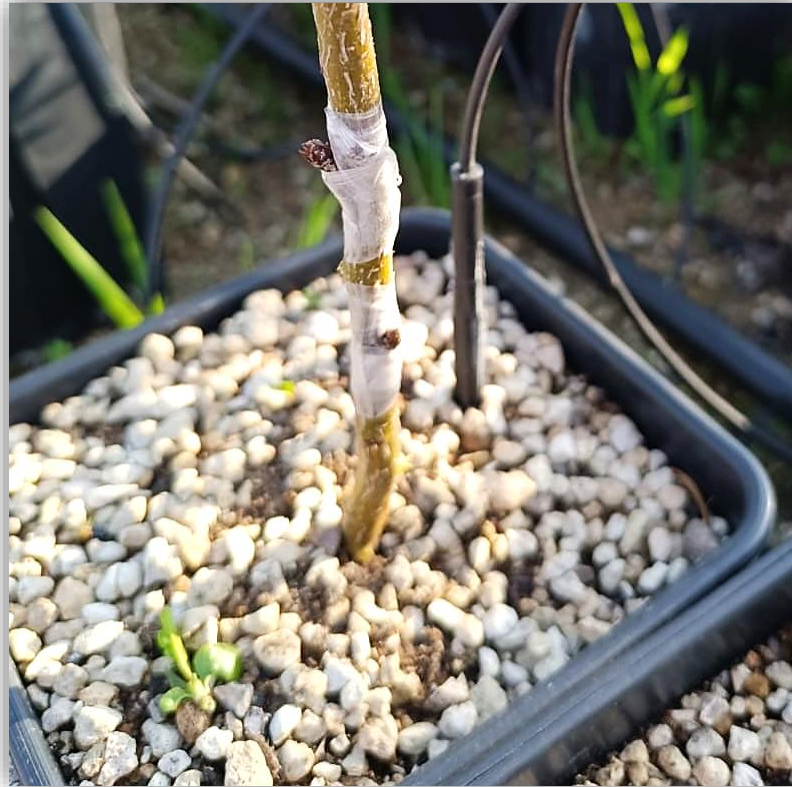
Cooling of plant material in room-temperature water

Acclimation of plant material at room-temperature for 12-24 h



➤ **After the thermal treatment:**

- bare-root plants were potted;
- scions were grafted onto rootstocks and potted as well;
- cuttings were placed in flasks containing water to evaluate their budburst.



➤ **Measured parameters:**

- **Budburst (%)** → bare-root plants and cuttings
- **Graft success rate (%)** → grafted plants

RESULTS

1. Bare-root plants:

- *J. regia* and *M. alba*: sprouting (%) comparable to untreated control, even at 50°C/45 min;
- *F. carica* and GF677: reduced sprouting (%) at 50°C/45 min;
- *P. dulcis*: highest susceptibility to HWT at both temperatures tested.

2025

SPECIES	CULTIVAR	TREATMENT	BUDBURST (%)
<i>Ficus carica</i>	Terlizzi	Untreated check	100.0 a
<i>Ficus carica</i>	Terlizzi	HWT 45°C-45 min	100.0 a
<i>Ficus carica</i>	Terlizzi	HWT 48°C-45 min	92.9 a
<i>Ficus carica</i>	Dottato	Untreated check	100.0 a
<i>Ficus carica</i>	Dottato	HWT 45°C-45 min	100.0 a
<i>Ficus carica</i>	Dottato	HWT 48°C-45 min	100.0 a
GF 677		Untreated check	100.0 a
GF 677		HWT 45°C-45 min	93.3 a
GF 677		HWT 48°C-45 min	62.5 b
<i>Juglans regia</i>	Sorrento	Untreated check	100.0 a
<i>Juglans regia</i>	Sorrento	HWT 45°C-45 min	100.0 a
<i>Juglans regia</i>	Sorrento	HWT 48°C-45 min	100.0 a
<i>Morus alba</i>	Bianco	Untreated check	100.0 a
<i>Morus alba</i>	Bianco	HWT 45°C-45 min	100.0 a
<i>Morus alba</i>	Bianco	HWT 48°C-45 min	100.0 a
<i>Prunus dulcis</i>	Tuono	Untreated check	47.0 a
<i>Prunus dulcis</i>	Tuono	HWT 45°C-45 min	12.5 ab
<i>Prunus dulcis</i>	Tuono	HWT 48°C-45 min	0.0 b
<i>Prunus dulcis</i>	Genco	Untreated check	61.1 a
<i>Prunus dulcis</i>	Genco	HWT 45°C-45 min	50.0 a
<i>Prunus dulcis</i>	Genco	HWT 48°C-45 min	5.9 b

2026

SPECIES	CULTIVAR	TREATMENT	BUDBURST (%)
<i>Ficus carica</i>	Terlizzi	Untreated check	100.0 a
<i>Ficus carica</i>	Terlizzi	HWT 48°C-45 min	96.0 a
<i>Ficus carica</i>	Terlizzi	HWT 50°C-45 min	68.0 b
<i>Ficus carica</i>	Dottato	Untreated check	100.0 a
<i>Ficus carica</i>	Dottato	HWT 48°C-45 min	100.0 a
<i>Ficus carica</i>	Dottato	HWT 50°C-45 min	48.0 b
GF 677		Untreated check	75.0 a
GF 677		HWT 48°C-45 min	72.7 a
GF 677		HWT 50°C-45 min	0.0 b
<i>Juglans regia</i>	Sorrento	Untreated check	100.0 a
<i>Juglans regia</i>	Sorrento	HWT 48°C-45 min	100.0 a
<i>Juglans regia</i>	Sorrento	HWT 50°C-45 min	100.0 a
<i>Morus alba</i>	Bianco	Untreated check	100.0 a
<i>Morus alba</i>	Bianco	HWT 48°C-45 min	96.0 a
<i>Morus alba</i>	Bianco	HWT 50°C-45 min	96.0 a
<i>Prunus dulcis</i>	Tuono	Untreated check	78.3 a
<i>Prunus dulcis</i>	Tuono	HWT 48°C-45 min	28.0 b
<i>Prunus dulcis</i>	Tuono	HWT 50°C-45 min	32.0 b
<i>Prunus dulcis</i>	Genco	Untreated check	88.0 a
<i>Prunus dulcis</i>	Genco	HWT 48°C-45 min	76.0 ab
<i>Prunus dulcis</i>	Genco	HWT 50°C-45 min	48.0 b

J. regia

M. alba

F. carica

GF677



Untreated check

HWT 48°C-45 min

HWT 50°C-45 min

2. Cuttings:

- *J. regia*: high budburst rate even at 50°C-45 min;
- *Morus* spp.: Response to HWT was cultivar-dependent; late cultivars (e.g., ‘Rosa’) tolerated treatments up to 50°C/45 min, whereas early-maturing cultivars maintained sprouting rates comparable to the untreated control only up to 48°C/45 min.

2025

SPECIES	CULTIVAR	TREATMENT	BUDBURST (%)
<i>Ficus carica</i>	Dottato	Untreated check	80.0 a
<i>Ficus carica</i>	Dottato	HWT 45°C-45 min	90.0 a
<i>Ficus carica</i>	Dottato	HWT 48°C-45 min	70.0 a
<i>Ficus carica</i>	Nero di Terlizzi	Untreated check	70.0 a
<i>Ficus carica</i>	Nero di Terlizzi	HWT 45°C-45 min	80.0 a
<i>Ficus carica</i>	Nero di Terlizzi	HWT 48°C-45 min	70.0 a

2026

SPECIES	CULTIVAR	TREATMENT	BUDBURST (%)
<i>Juglans regia</i>	Chandler	Untreated check	100.0 a
<i>Juglans regia</i>	Chandler	HWT 48°C-45 min	100.0 a
<i>Juglans regia</i>	Chandler	HWT 50°C-45 min	100.0 a
<i>Morus</i> spp.	Alba	Untreated check	68.0 a
<i>Morus</i> spp.	Alba	HWT 48°C-45 min	56.0 a
<i>Morus</i> spp.	Alba	HWT 50°C-45 min	36.0 b
<i>Morus</i> spp.	Bianco	Untreated check	72.0 a
<i>Morus</i> spp.	Bianco	HWT 48°C-45 min	52.0 a
<i>Morus</i> spp.	Bianco	HWT 50°C-45 min	24.0 b
<i>Morus</i> spp.	Rosa	Untreated check	80.0 a
<i>Morus</i> spp.	Rosa	HWT 48°C-45 min	80.0 a
<i>Morus</i> spp.	Rosa	HWT 50°C-45 min	90.0 a

3. Grafted plants:

- *P. dulcis* and *P. avium*: Grafting success was not affected by the 48°C/45 min treatment; although vegetative recovery was delayed and shoot length was reduced. The 50°C/45 min completely inhibited grafting success.

2025

SPECIES	CULTIVAR	TREATMENT	GRAFT SUCCESS RATE (%)
<i>Prunus dulcis</i>	Genco	Untreated check	93.8 a
<i>Prunus dulcis</i>	Genco	HWT 45°C-45 min	100.0 a
<i>Prunus dulcis</i>	Genco	HWT 48°C-45 min	80.0 a
<i>Prunus avium</i>	Ferrovia	Untreated check	66.7 a
<i>Prunus avium</i>	Ferrovia	HWT 45°C-45 min	50.0 a
<i>Prunus avium</i>	Ferrovia	HWT 48°C-45 min	33.3 a

2026

SPECIES	CULTIVAR	TREATMENT	GRAFT SUCCESS RATE (%)
<i>Prunus dulcis</i>	Genco	Untreated check	92.0 a
<i>Prunus dulcis</i>	Genco	HWT 48°C-45 min	70.8 a
<i>Prunus dulcis</i>	Genco	HWT 50°C-45 min	0.0 b
<i>Prunus avium</i>	Ferrovia	Untreated check	95.5 a
<i>Prunus avium</i>	Ferrovia	HWT 48°C-45 min	87.5 a
<i>Prunus avium</i>	Ferrovia	HWT 50°C-45 min	0.0 b



P. avium

P. dulcis



- **The response to hot-water treatment (HWT)** strongly depends on the species, cultivar and type of propagation material;
- **Protocol at 48°C for 45 min:** showed good compatibility with most material;
- **Protocol at 50°C for 45 min:**
 - generally resulted in a substantial reduction in viability, particularly in scion wood and rootstocks (GF677 and *P. mahaleb*);
 - the main exception are bare-root mulberry and walnut plants maintained high bud-break percentages even under more severe thermal conditions;



TREATMENTS ON INFECTED MATERIALS:

GRAPE: XFF

ALMOND: XFF & XFM



Hot water treatments on grape cuttings:

HWT_45°C for 60 min

- Bacterial cells survived
- Positive isolation *in vitro*
- Positive leaves grown on cuttings

HWT_47°C for 60 min

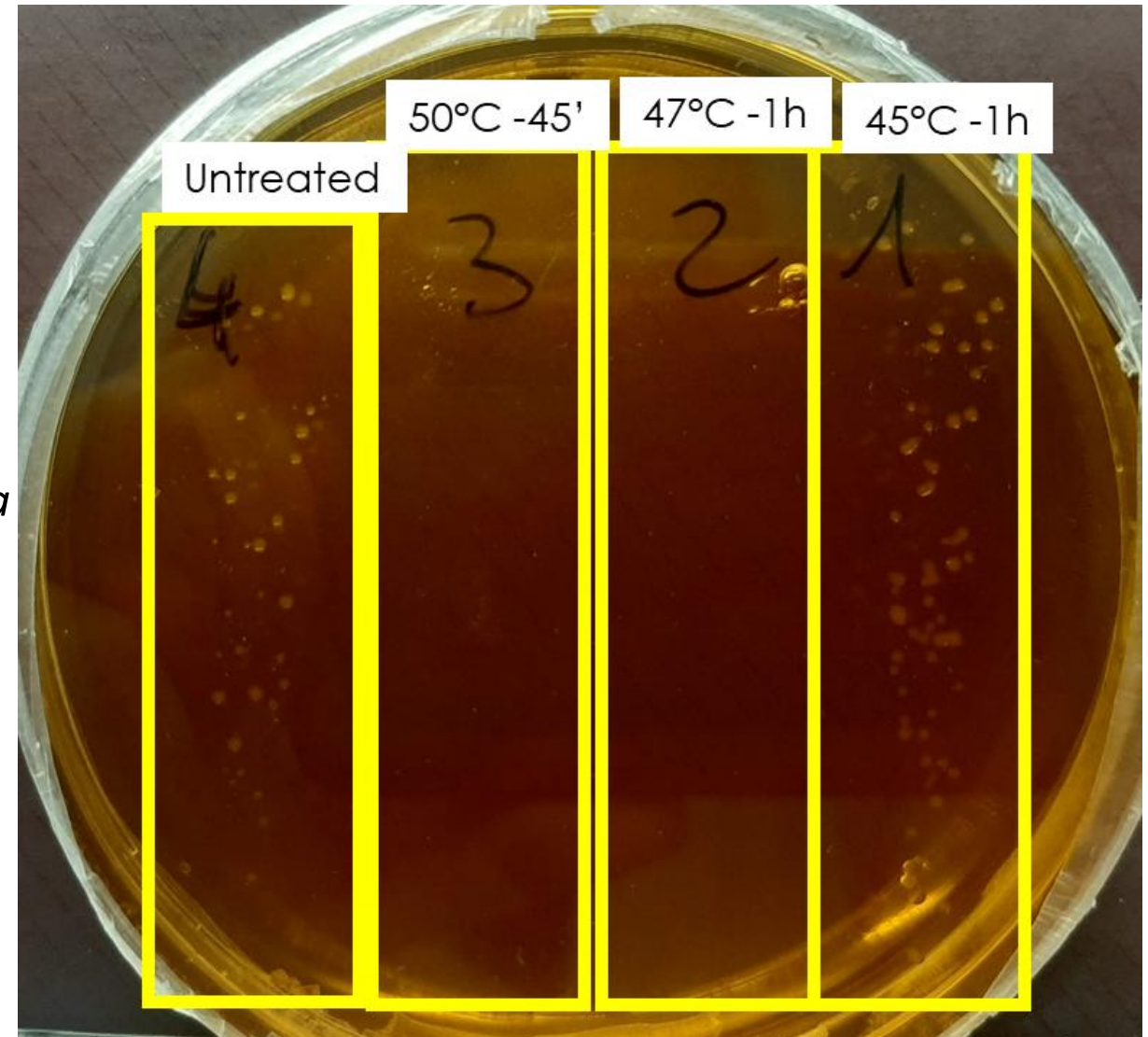
- No colonies recovered *in vitro*
- However, qPCR detected bacterial spread *in planta*

48°C for 60 min

- No colonies recovered *in vitro*
- No bacterial detection in new leaves

50°C for 45 min (official protocol)

- No colonies recovered
- No bacterial movement to new leaves



Hot water treatments on grape cuttings:

HWT_45°C for 60 min

- Bacterial cells survived
- Positive isolation *in vitro*
- Positive leaves grown on cuttings



HWT_47°C for 60 min

- No colonies recovered *in vitro*
- However, qPCR detected bacterial spread *in planta*



48°C for 60 min

- No colonies recovered *in vitro*
- No bacterial detection in new leaves



50°C for 45 min (official protocol)

- No colonies recovered
- No bacterial movement to new leaves



Thermal treatments on almond shoots:



47°C x 60 min

48°C x 30 min

50°C x 30 min

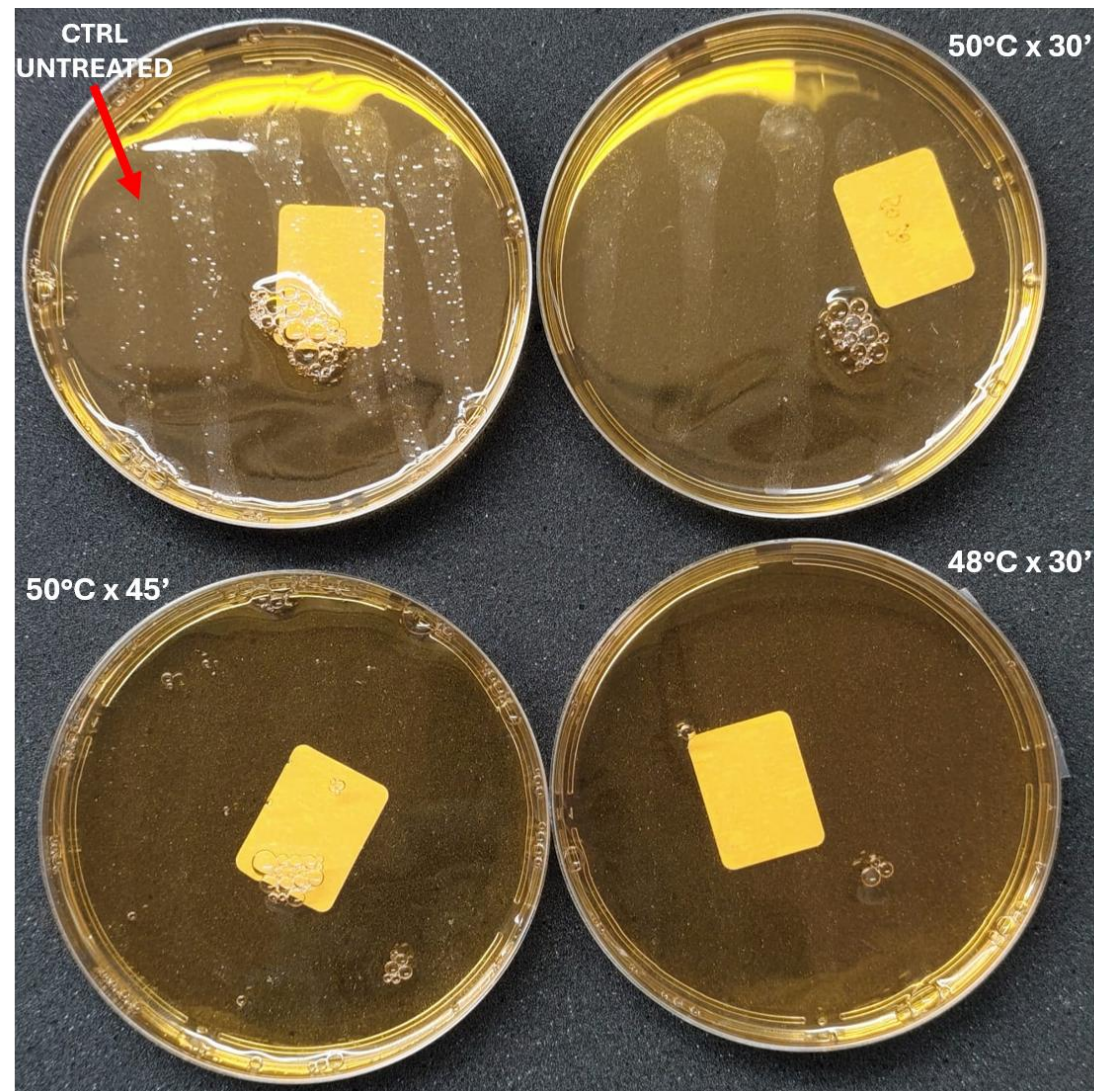
50°C x 45 min

47°C x 45 min

48°C x 30 min

49°C x 30 min

50°C x 45 min





CONCLUSION

Thermotherapy as a phytosanitary tool against *Xyella fastidiosa*

- Hot water treatment (50°C for 45 min) is currently the most robust and validated protocol for the sanitation of dormant grapevine propagation material. Preliminary evidence suggests that the protocol could be extended to other deciduous woody hosts, including walnuts and mulberry.
 - The approach offers a sustainable, chemical-free strategy to reduce the risk of long-distance spreading of *Xylella fastidiosa* through nursery stock.
 - Further research is needed to optimize treatment conditions, assess host-specific tolerance and validate alternative heat-based technologies (e.g. steam)
- 



**Thank you for
your attention**





Xylella fastidiosa

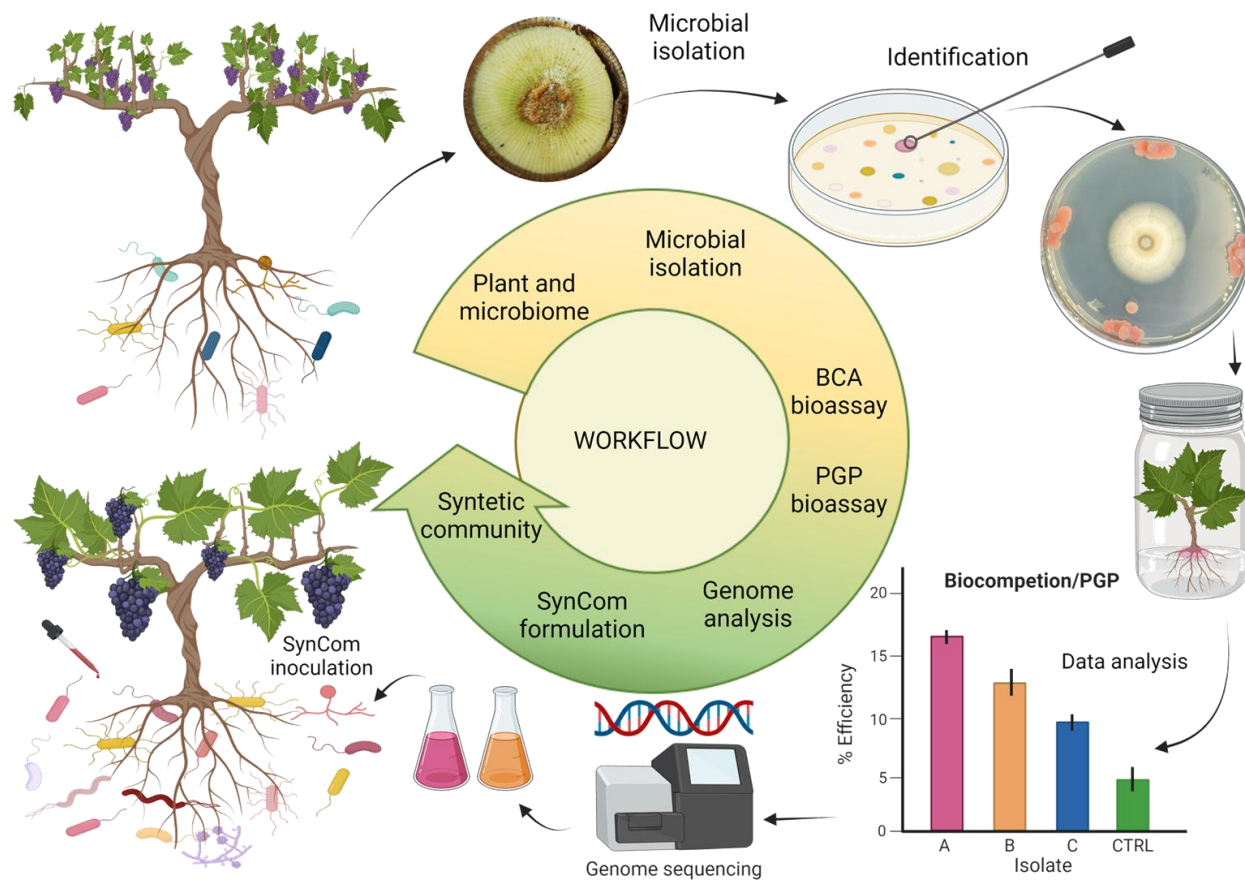
5th European conference

Bari, Italy | 22-26 June 2026



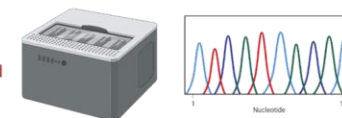
REGIONE PUGLIA

FUNCTIONAL SCREENING OF BACTERIAL ISOLATES REVEALS CANDIDATES FOR THE BIOLOGICAL CONTROL OF *XYLELLA FASTIDIOSA* SUBSP. *FASTIDIOSA* IN GRAPEVINE



Focus on microbe features

Metagenomics
To depict the microbial biodiversity and its associated functional biodiversity



Metabolomics
Microbes exudates and quorum sensing compounds

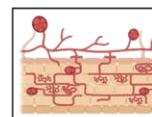


Volatilomics
Microbes volatile compounds (VOCs)



Selection of the core microbiota

Characterization of PGP & BCA



Focus on plant features

Metatranscriptomics
To uncover plant responses during the interactions

Metabolomics
Plant root exudates

Volatilomics
Plant volatile compounds (VOCs)

Phenomics in response to SynComs application
Evaluation of agronomic traits (above and below ground) and spectranomics

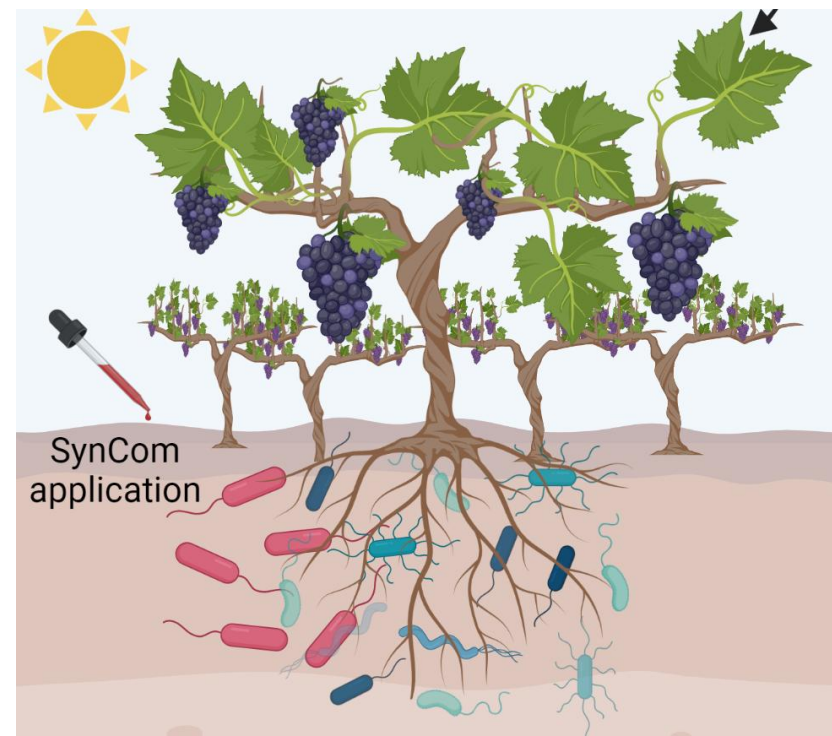
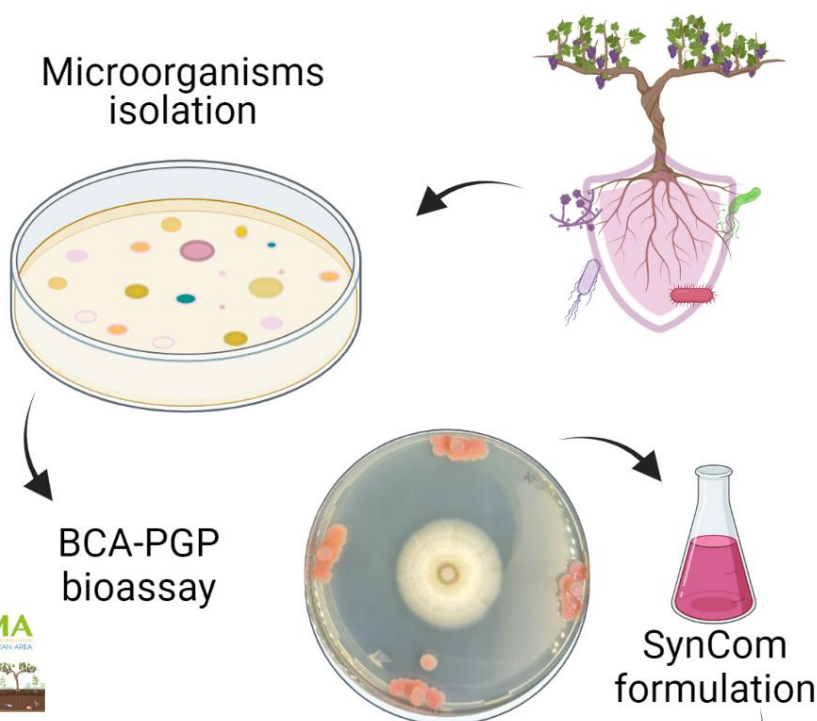


OUR MICROBIAL GRAPEVINE BANK

We started by developing **a collection of plant endophytes**, specifically exploiting plants from challenging conditions or that are already adapted to stressful conditions.

Microbes were isolated on SPM medium, from samples of woody tissues of important varieties (Glera, Nebbiolo, Barbera, Garganega, Tocai, ecc...) and rootstocks (110R, 140RU, K5BB, ecc...).

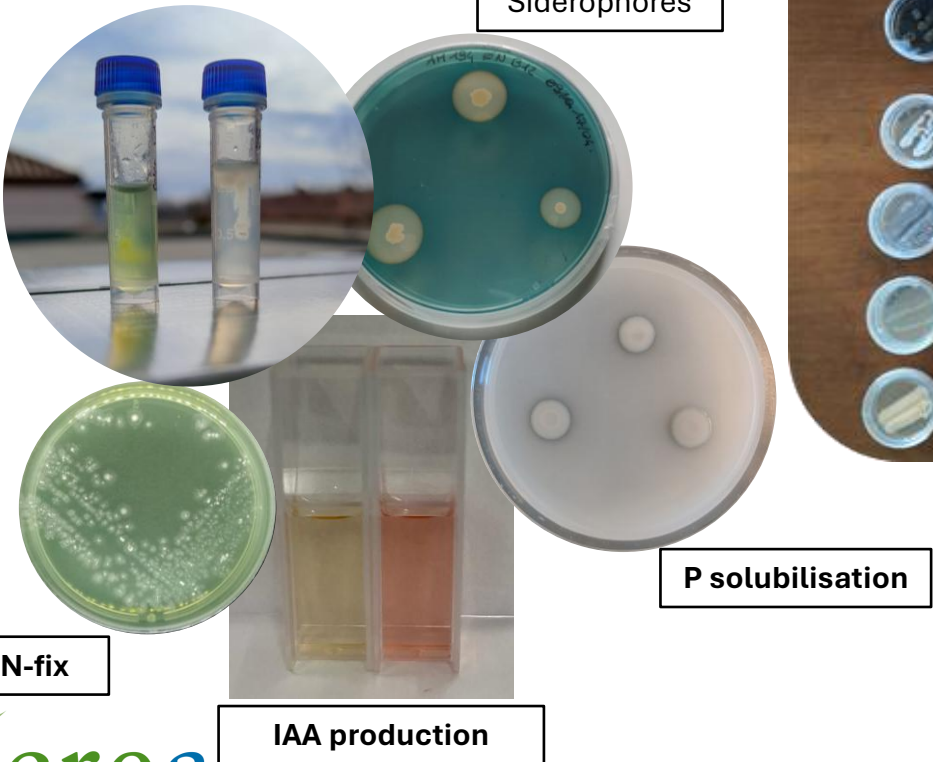
A long journey since 8 years!



SELECTING THE MICROBIAL ISOLATES

To date, **more than 1,000 isolates** have been obtained and included in the **ViMED-Biomebank** collection.

Plant Growth Promoting traits



Among these isolates, more than 200 have already been evaluated for plant growth-promoting traits. This preliminary screening allowed the selection of approximately 100 isolates displaying characteristics of interest, which have been further tested for their ability to inhibit *Xylella* growth.

Biocontrol

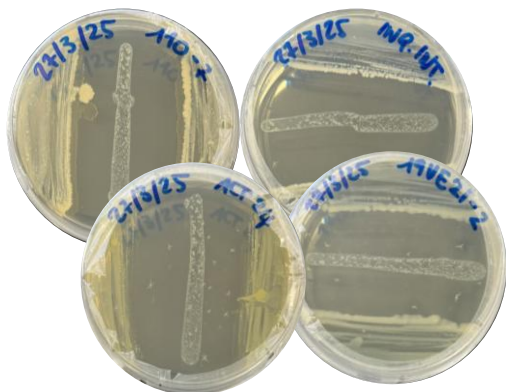
Phosphate solubilization
IAA production
ACC degradation
Siderophore production
...

IN-VITRO BIOCONTROL ASSAY

Control



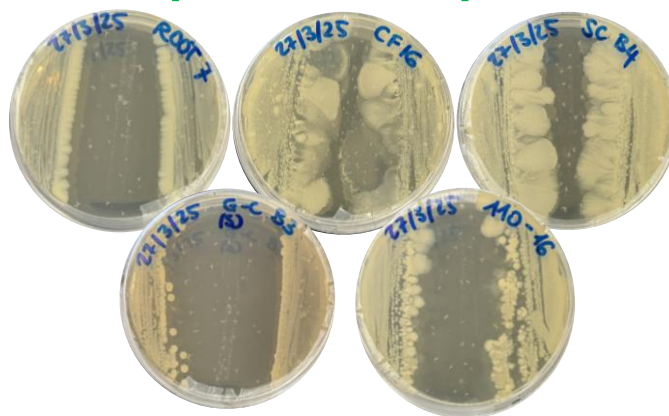
Ineffective
(129 isolates)



Successful biocontrol



Potential biocontrol
(9 isolates)



First screening

138 microorganisms tested

High biocontrol activity

27 microorganisms

Nocardioides sp., *Serratia sp.*, *Microbacterium sp.*

Final screening

Ongoing

Mutual exclusion tests and
SynCom formulation

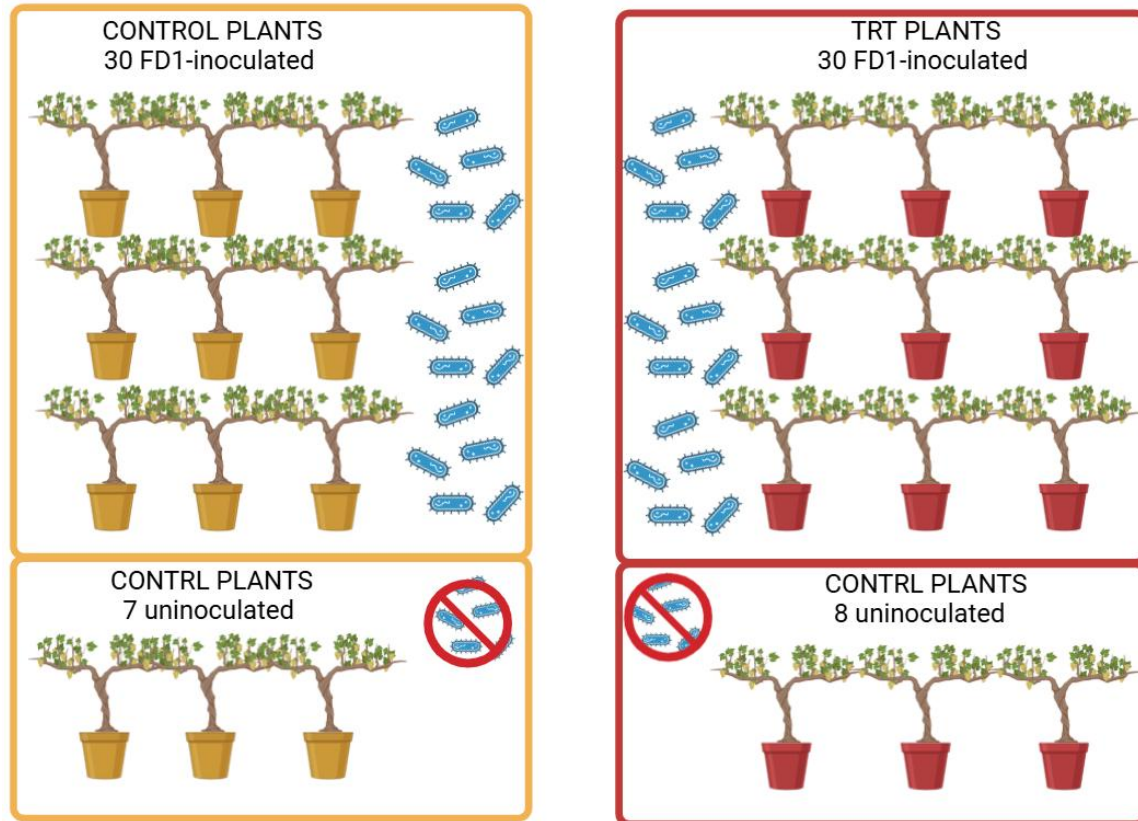
Next step

Plant inoculum

A stepwise screening strategy is being applied to identify bacterial strains with the **strongest antagonistic potential**. From the initial collection, the most promising candidates are progressively selected and characterized to **formulate compatible microbial consortia** for subsequent validation in grapevine plants.

IN-VIVO BIOCONTROL ASSAY

The plants were firstly inoculated in our laboratories (cutting stage) and subsequently re-inoculated with the microbial consortium 15 days after planting in Palma.



Xylella FD1 inoculation experiment:

- **CONTROL plants**, 30 out of the 37 plants inoculated with *Xylella* FD1, 7 kept as uninoculated controls.
- **TRT plants**, 30 out of the 38 plants inoculated with *Xylella* FD1, 8 kept as uninoculated controls.



Diego Olmo and Eduardo Moralejo

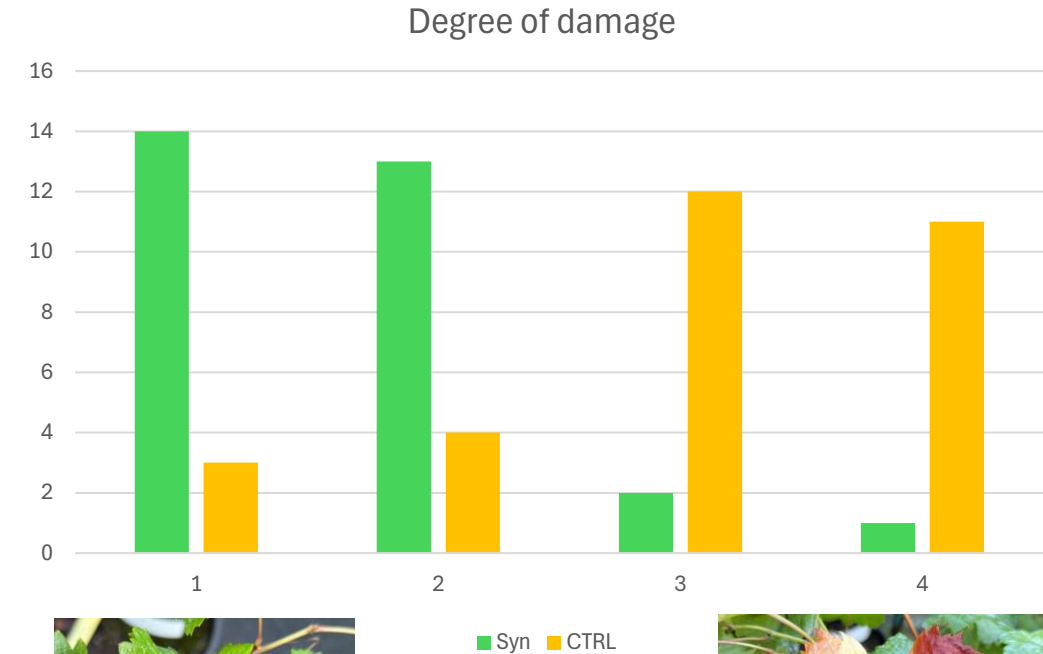
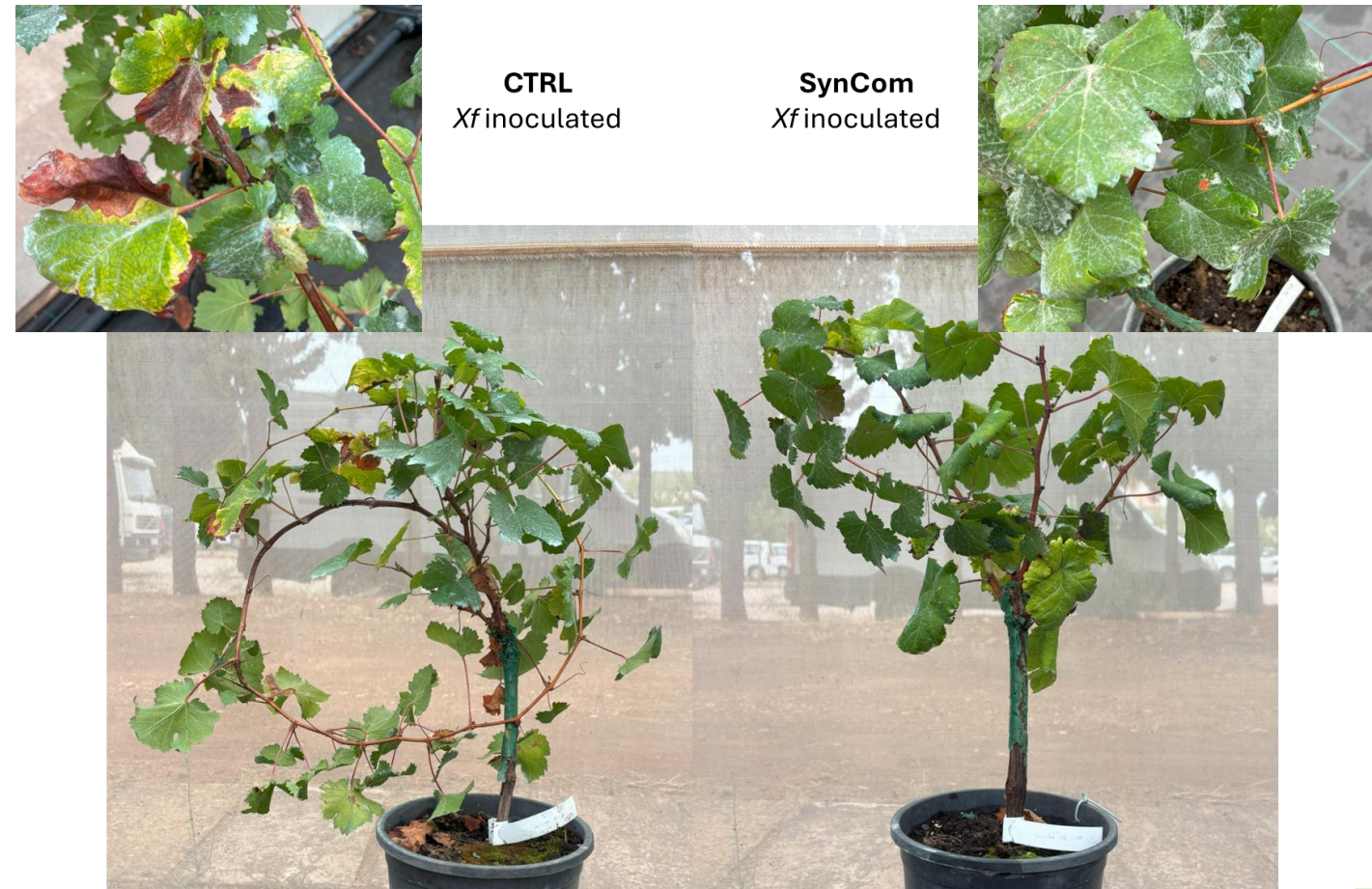


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Agroalimentària i Pesquera

FIRST RESULTS: DISEASE ALLEVIATION

The plants were firstly inoculated in our laboratories (cutting stage) and subsequently re-inoculated with the microbial consortium 15 days after planting in Palma.



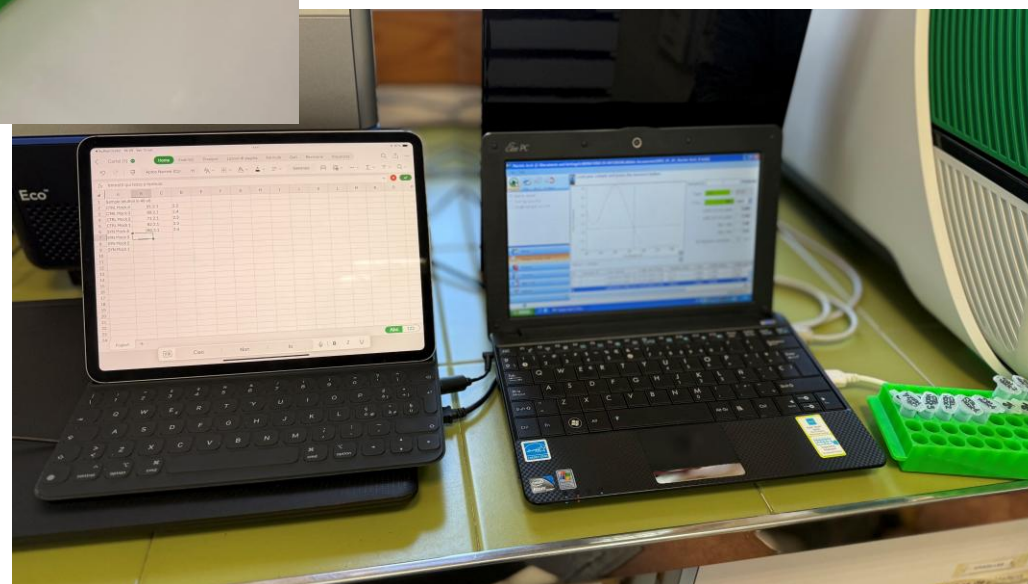
Treatment	I leaf	II leaf	Upper	Lower	Total	Match petiole
Syn	59%	56%	1,4800	1,1600	3,6400	15%
CTRL	86%	71%	4,8929	3,7143	10,1786	46%

MOLECULAR ANALYSES ONGOING...



DNA and RNA extraction from leaf petioles
using an accredited grapevine-specific method

Analysis of microbiome and transcriptome
responses

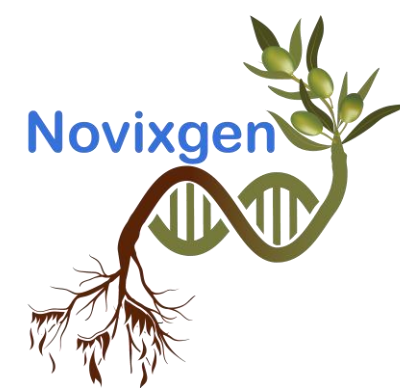
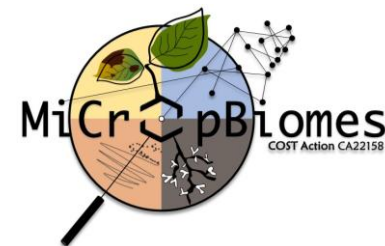


THANK YOU!

Micol Guaschino, Loredana Moffa, Francesco Favaretto, Valeria Scala, Stefania Loreti, Elena Santilli, Walter Chitarra

Diego Olmo, Eduardo Moralejo

Antonio Magaña



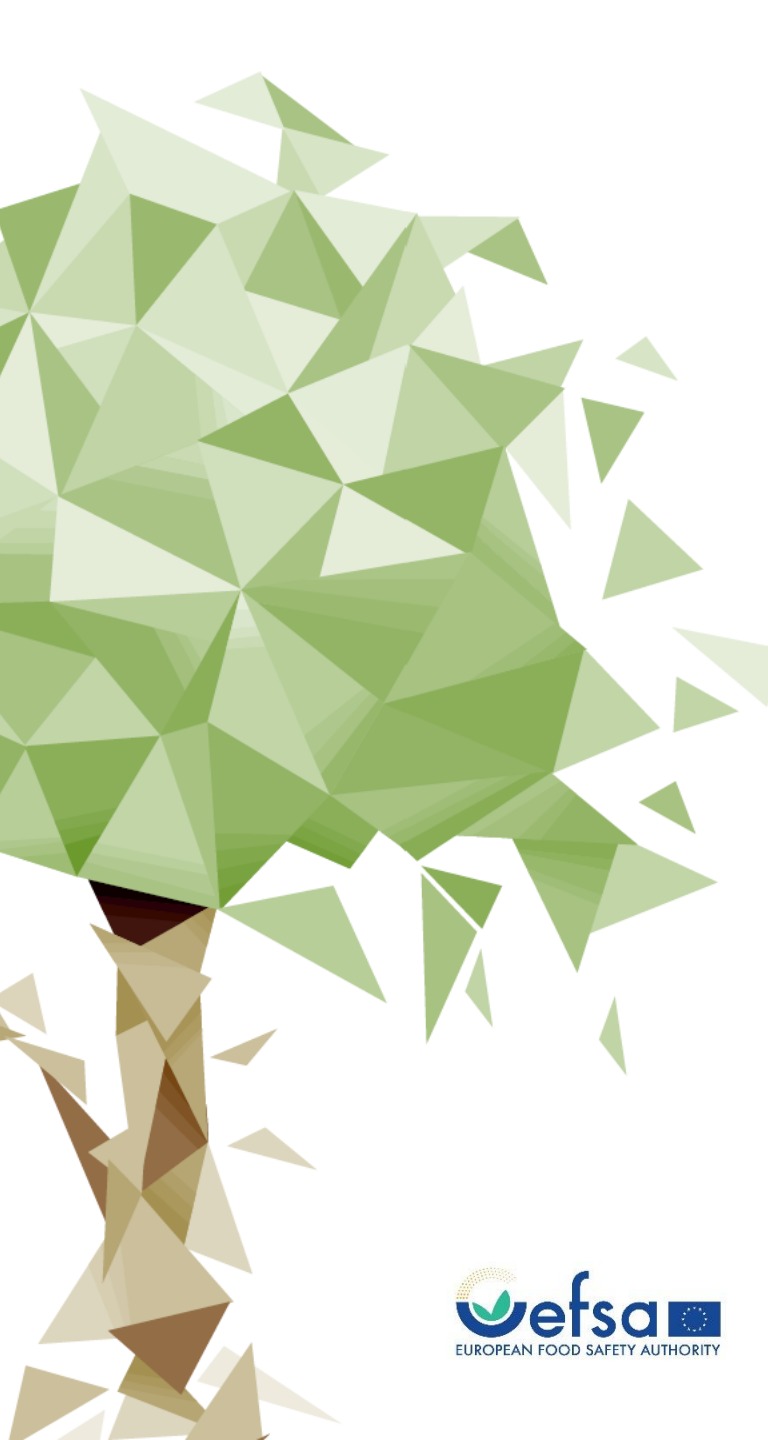
This research was funded
by NOVIXGEN project D.M.
MASAF n. D.M. N.0664890
del 29/12/2022



**Conselleria d'Agricultura,
Pesca i Medi Natural**

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Agroalimentària i Pesquera





Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026

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

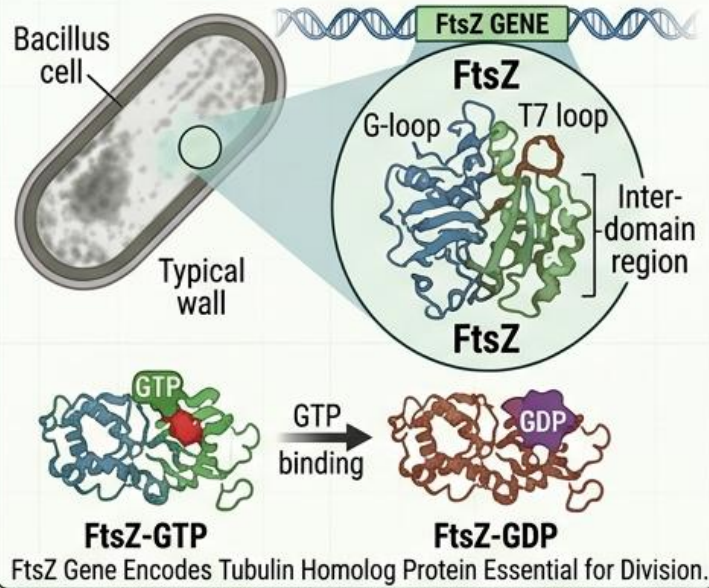
De Souza-Neto R. R., Astolfi A., Caliandro R., Del Grosso C., De Stradis A., Sabatini S., Amoia S. S., Caliandro R., Barreca M. L., Saldarelli P., Saponari M., and Giampetruzzi A.



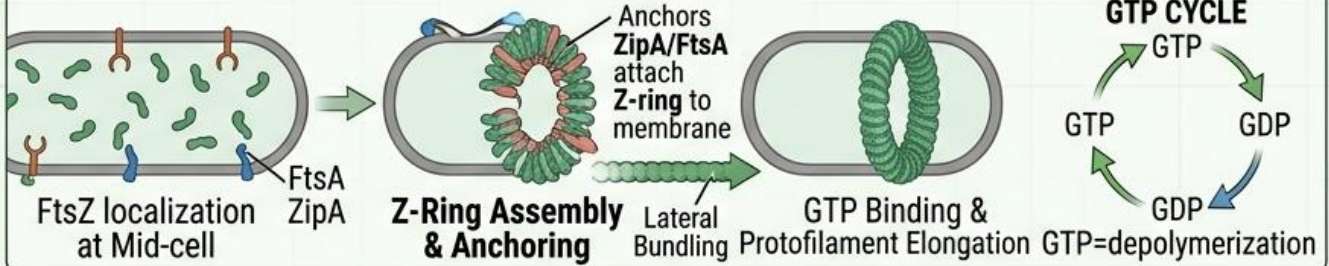
Introduction

FUNCTION OF FTSZ GENE IN BACTERIAL CYTOKINESIS

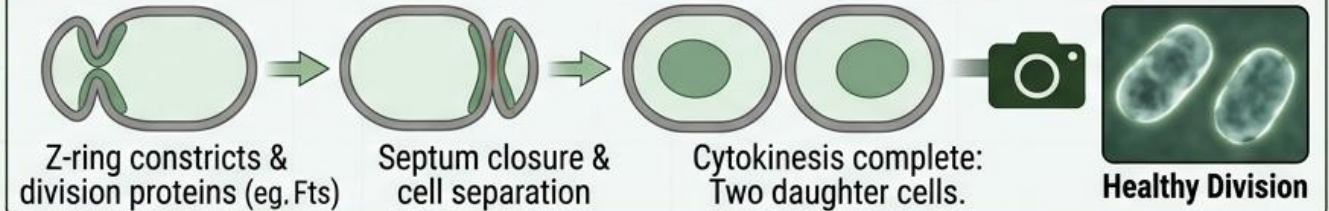
INTRODUCTION TO FTSZ GENE & PROTEIN



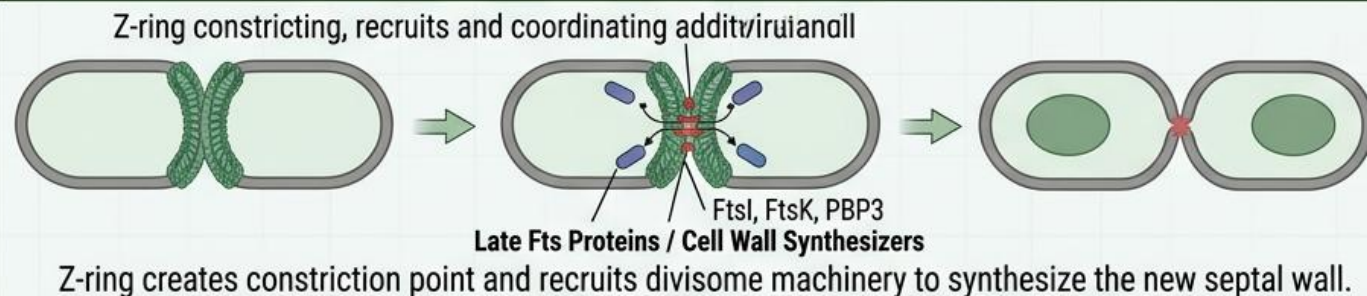
FTSZ DYNAMICS & "Z-RING" FORMATION



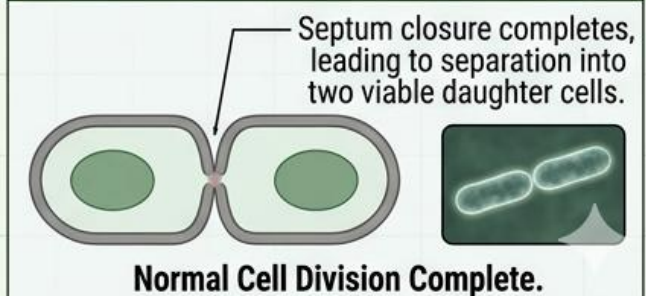
NORMAL CELL DIVISION PIPELINE



KEY ROLE: COORDINATING CYTOKINESIS



CELL SEPARATION PROCESS





Objectives

Identification of novel potential inhibitor molecules targeting *Xylella fastidiosa* FtsZ to control bacterial growth *in vitro*.



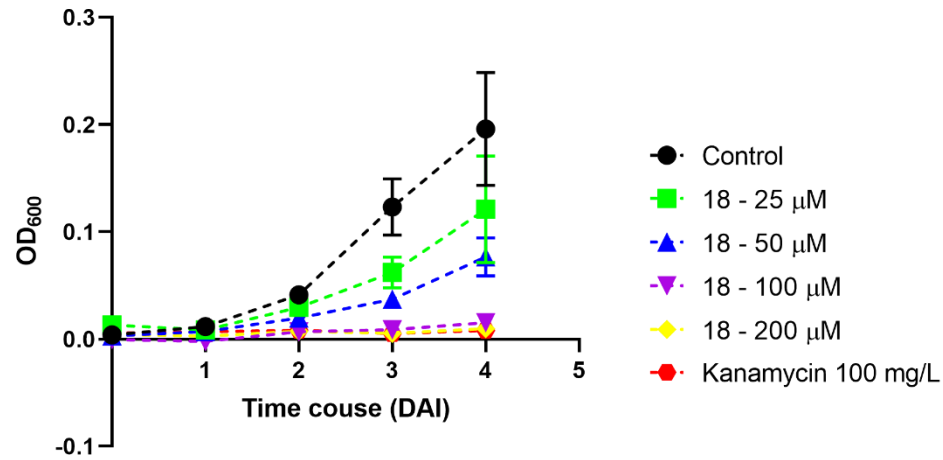


Screening of chemical analogues provided by UNIPG based on chemoinformatic analysis

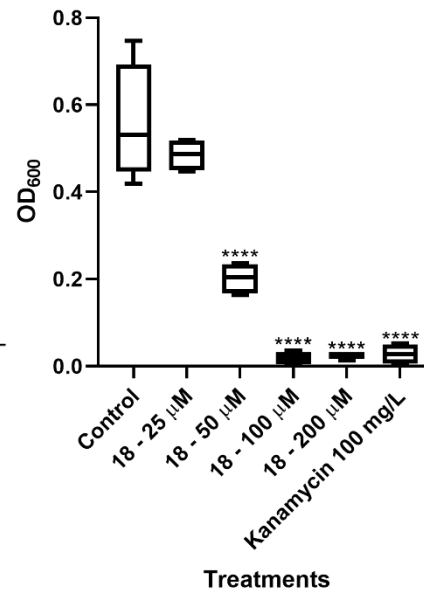
Compound	Planktonic growth	Dunnett's test	Biofilm growth	Dunnett's test
Control	0,069±0,015	-	0,4±0,17	-
1	0,014±0,013	****	0,034±0,053	****
2	-0,016±0,0049	****	0,058±0,063	****
3	0,2±0,02	****	0,14±0,028	**
4	0,05±0,013	n. s.	0,46±0,086	n. s.
5	0,12±0,0077	***	0,7±0,28	***
6	0,0082±0,0027	****	0,3±0,062	n. s.
7	0,34±0,014	****	0,31±0,058	n. s.
8	0,26±0,0091	****	0,57±0,19	n. s.
9	0,13±0,024	****	0,058±0,023	****
10	0,028±0,0072	**	0,37±0,042	n. s.
11	-0,23±0,0056	****	0,25±0,017	n. s.
12	0,017±0,011	**	0,36±0,088	n. s.
13	0,11±0,026	**	0,083±0,015	***
14	0,036±0,0033	n. s.	0,57±0,11	n. s.
15	0,3±0,075	****	0,12±0,043	***
16	0,17±0,008	****	0,42±0,23	n. s.
17	-0,045±0,0096	****	0,015±0,047	****
18	-0,0037±0,013	****	0,073±0,023	****
19	0,021±0,0053	***	0,18±0,12	*
20	-0,0033±0,0094	****	0,054±0,069	****
21	0,054±0,018	n. s.	0,12±0,042	**
22	0,14±0,016	****	0,16±0,01	*
Kanamycin (100 mg/L)	0,0093±0,011	****	0,04±0,028	****

Dose-response effect on *Xylella fastidiosa* subsp. *pauca*

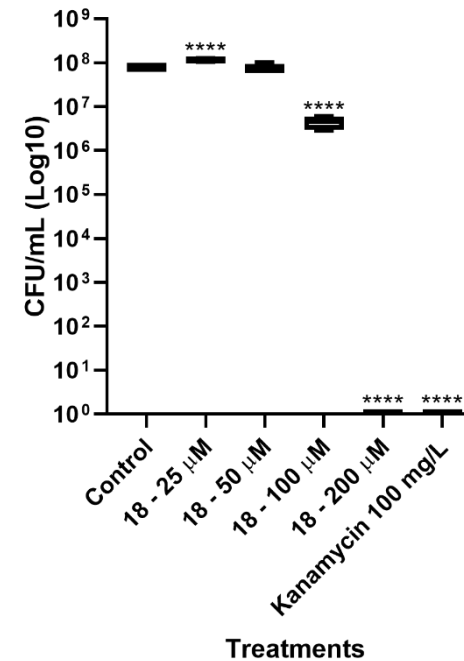
Growth curve



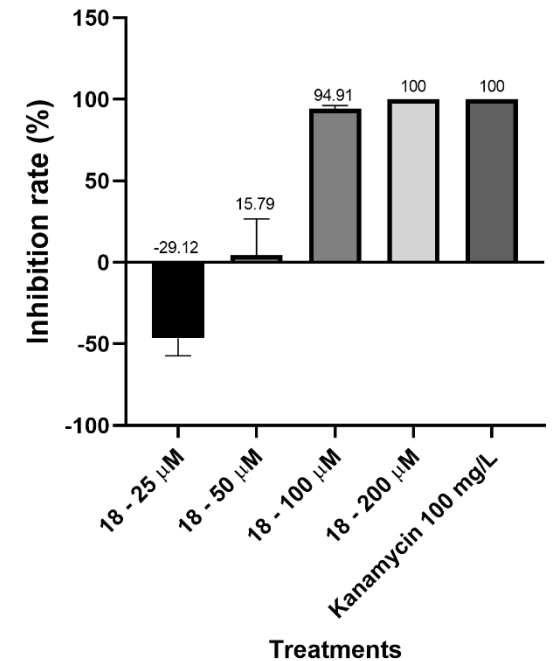
Biofilm



CFU

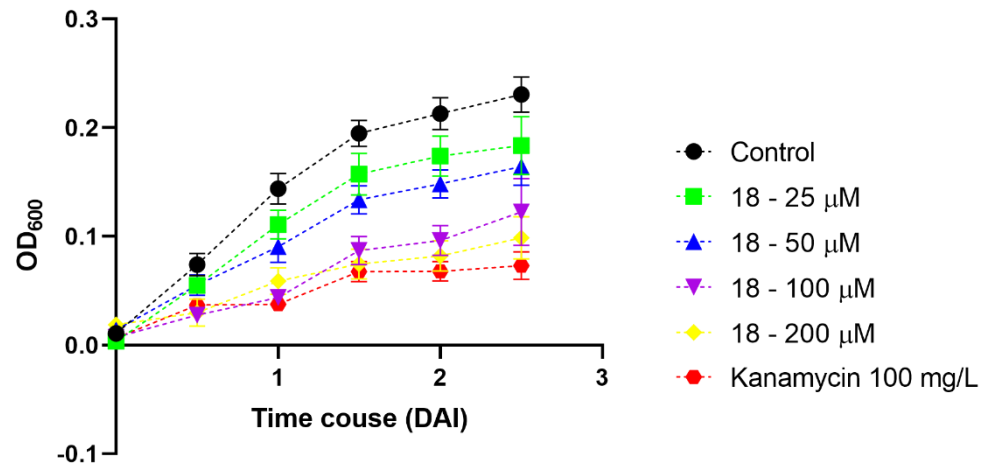


Inhibition rate

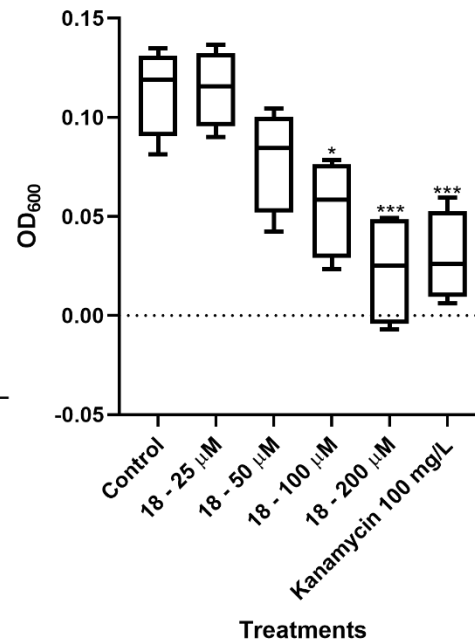


Dose-response effect on *Xylella fastidiosa* subsp. *fastidiosa*

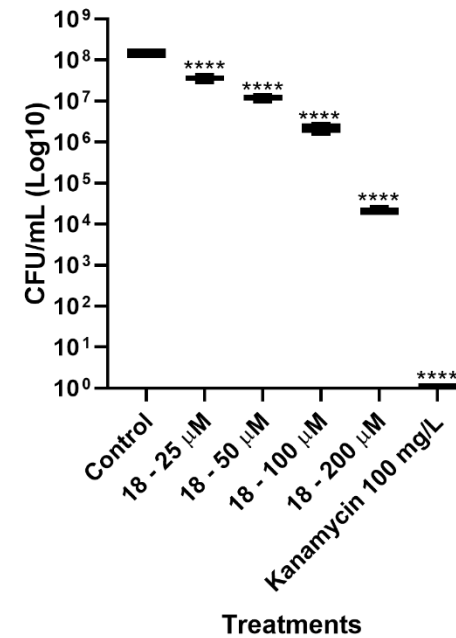
Growth curve



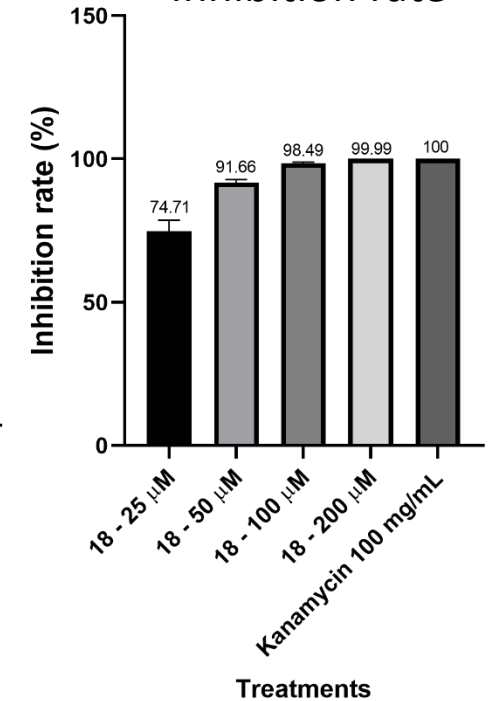
Biofilm



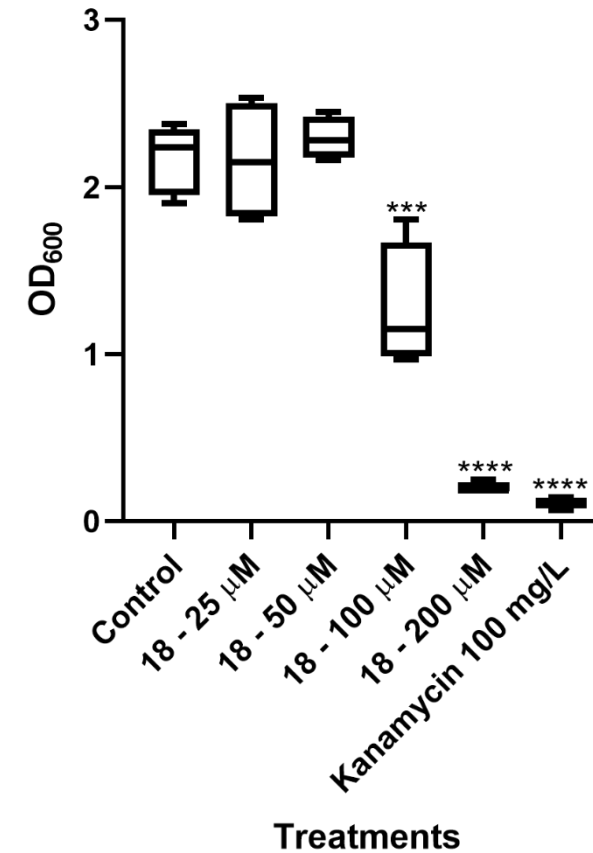
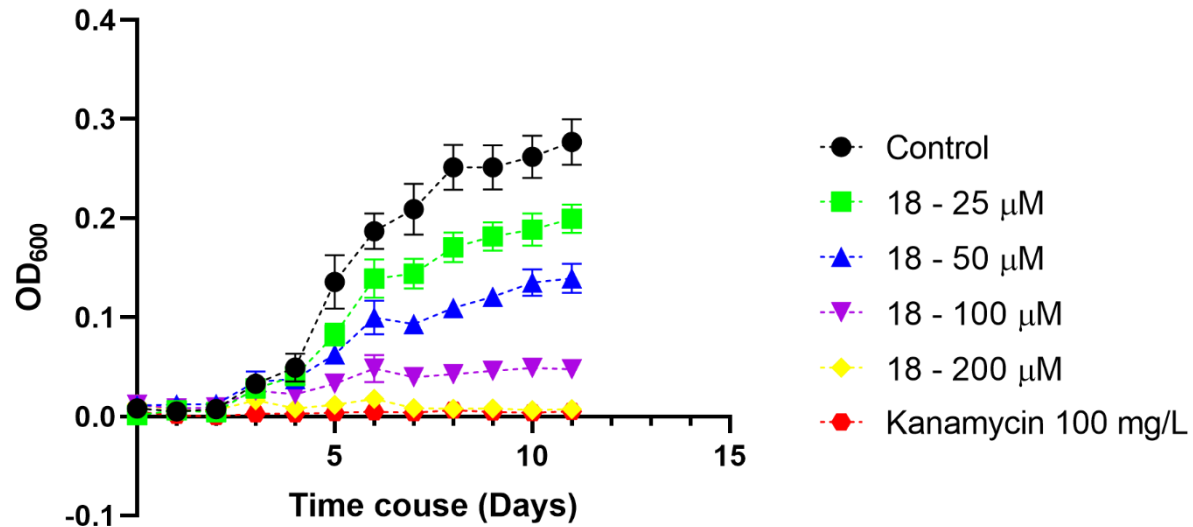
CFU



Inhibition rate



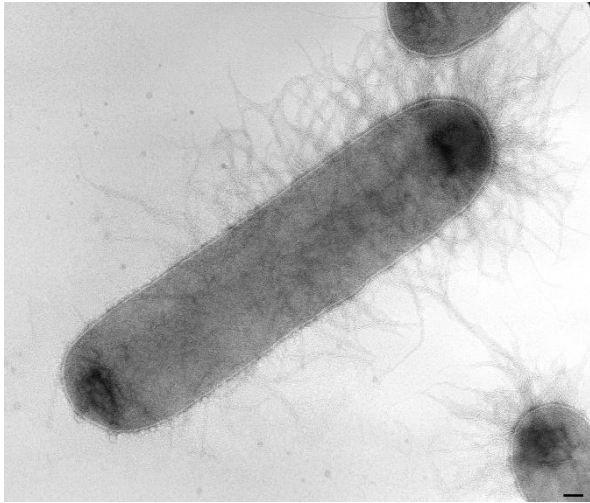
Dose-response effect on *Xylella fastidiosa* subsp. *multiplex*



Phenotyping assays

Transmission Electronic Microscopy (TEM)

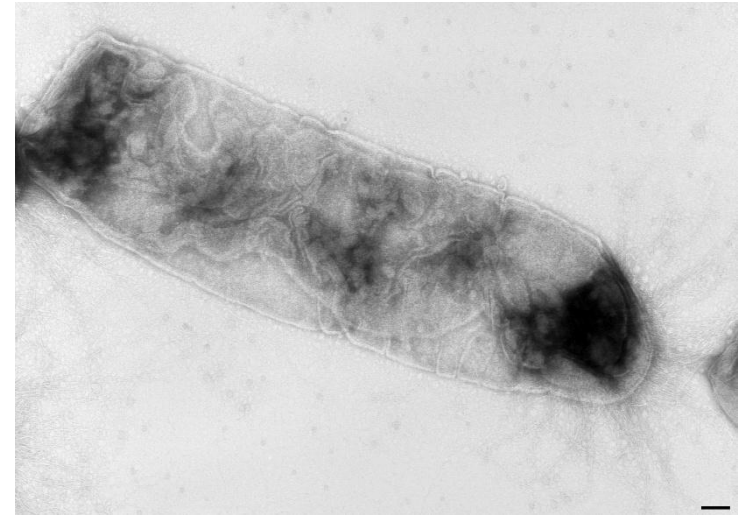
Control



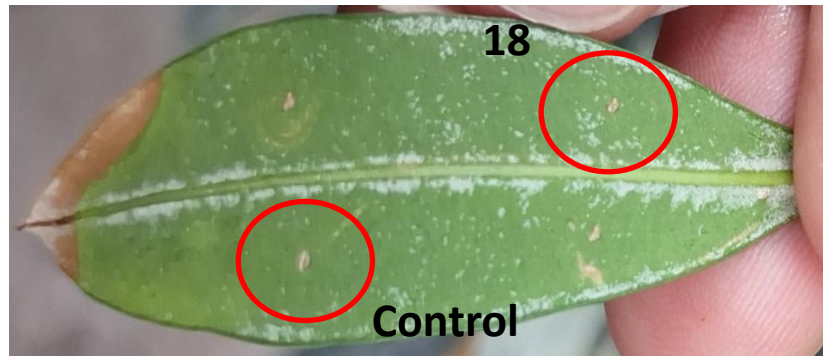
18 - 200 μ M



Kanamycin 100 mg/L



Phytotoxicity assay: 2 months after infiltration





Partial conclusions

These findings support FtsZ as a conserved and potentially druggable target in *X. fastidiosa* and identify compound 18 as a promising agrochemical candidate for the development of novel antibacterial approaches against *X. fastidiosa*.

You are invited to visit the poster #61 for more details



Acknowledgments

CNR – Institute for Sustainable Plant Protection

Dr. Annalisa Giampetruzzi
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University of Perugia

Dr. Maria Letizia Barreca
Dr. Stefano Sabatini
Dr. Andrea Astolfi

Thank you

Email: reinaldorodriguesdesouzaneto@cnr.it





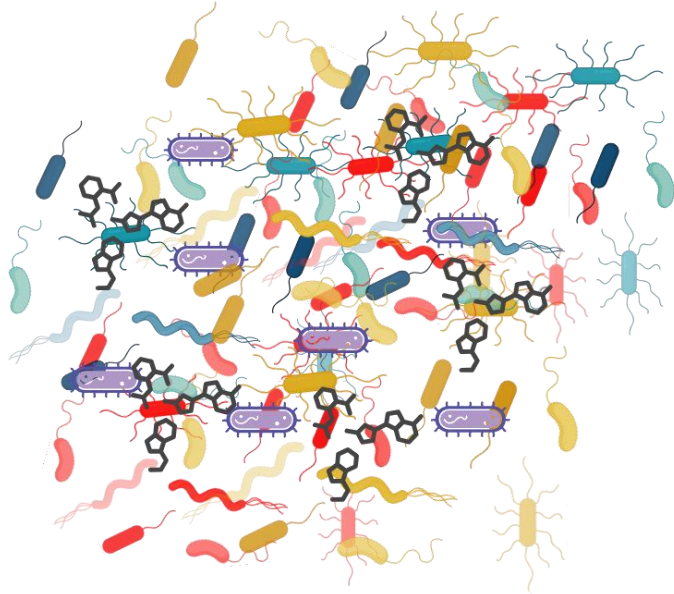
Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026

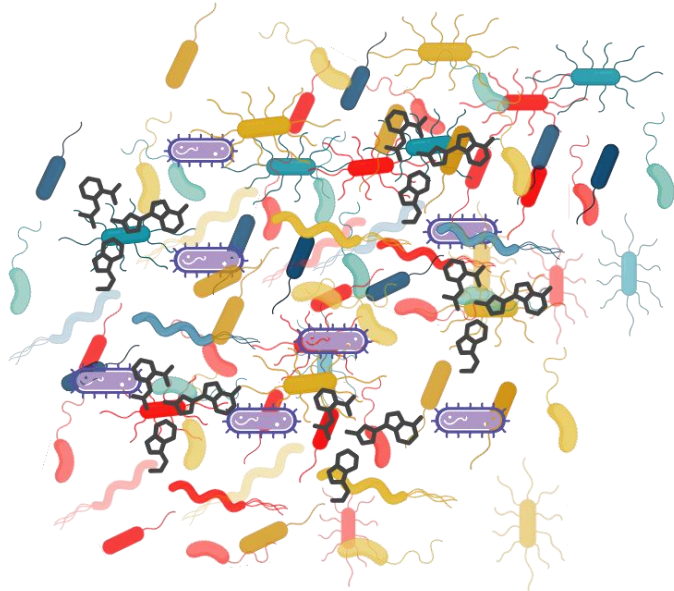


Microbial control of *Xylella fastidiosa* through a novel antagonistic protein from the plant phyllosphere

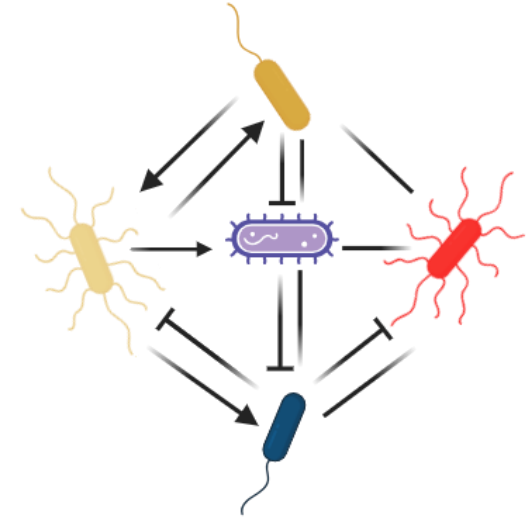


Bacterial ecology

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

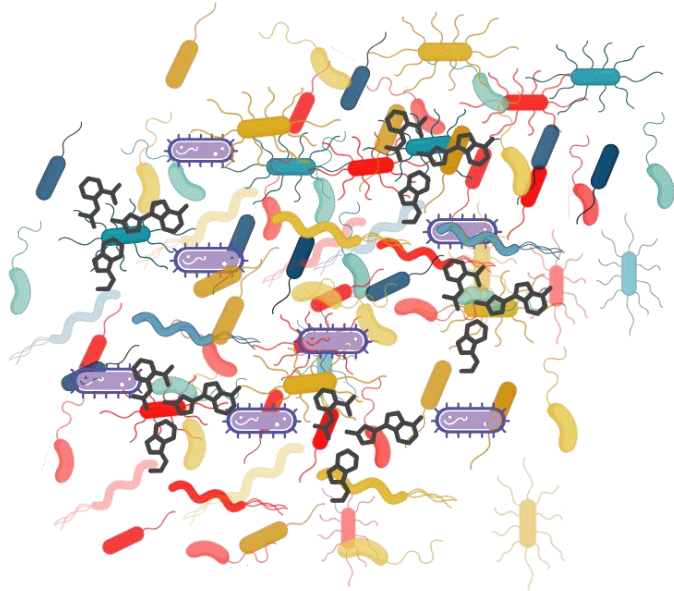


Bacterial ecology

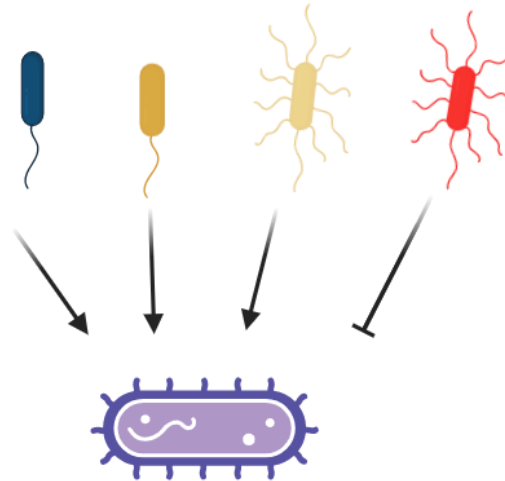


Inter-bacterial interactions

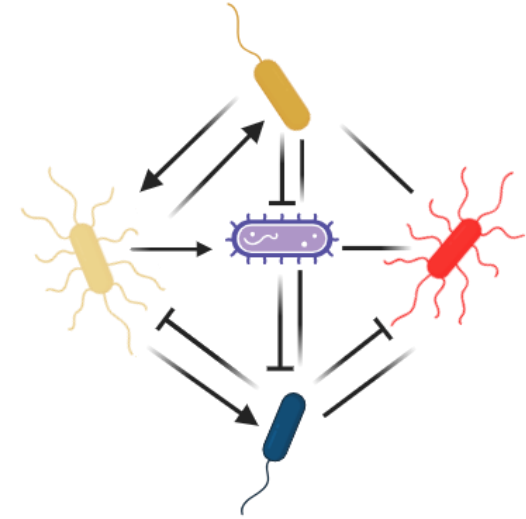
Microbial control of *Xylella fastidiosa* through a novel antagonistic protein from the plant phyllosphere



Bacterial ecology

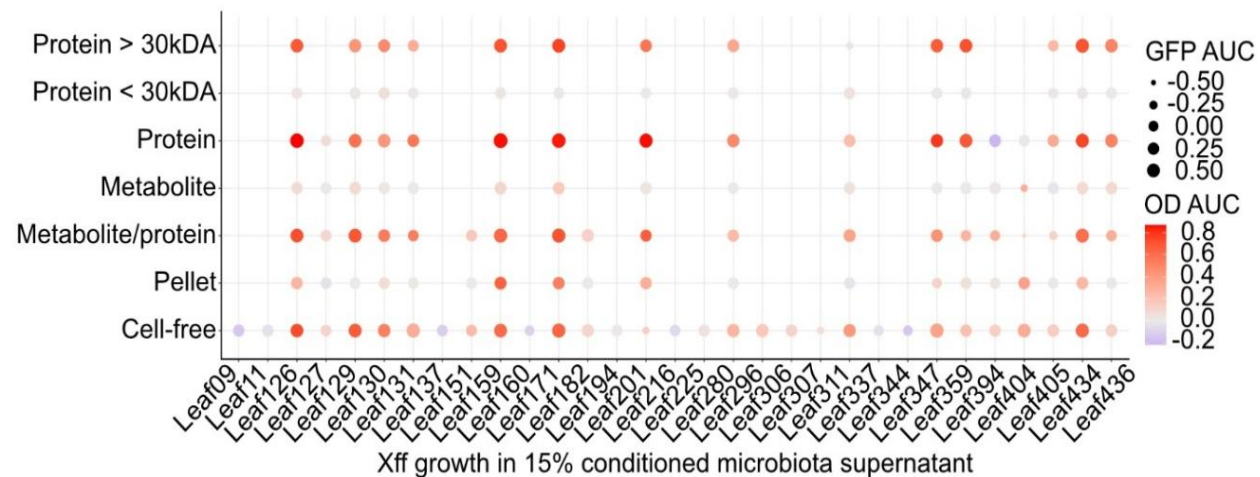


Inhibit the growth of *X. fastidiosa*

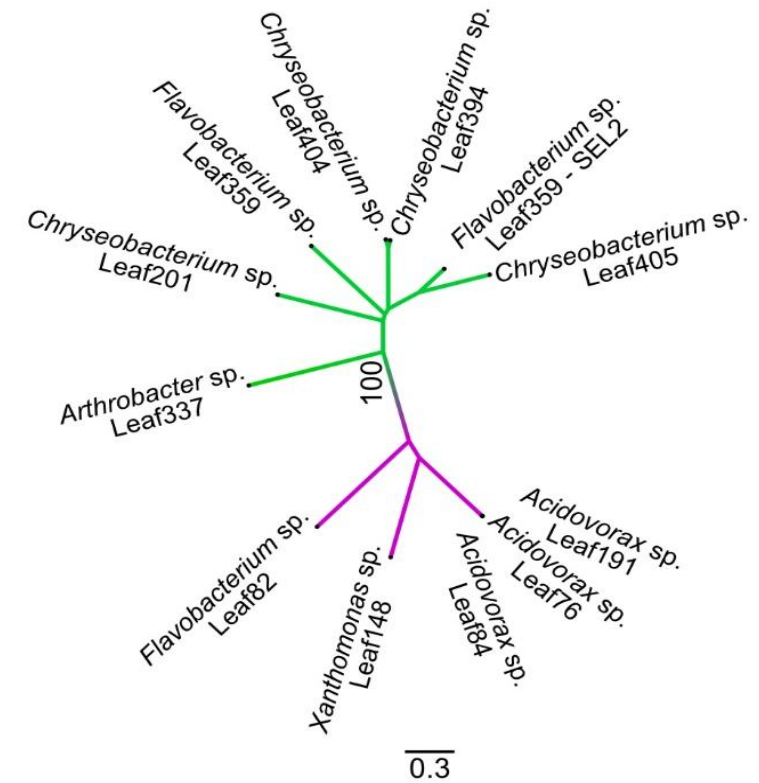
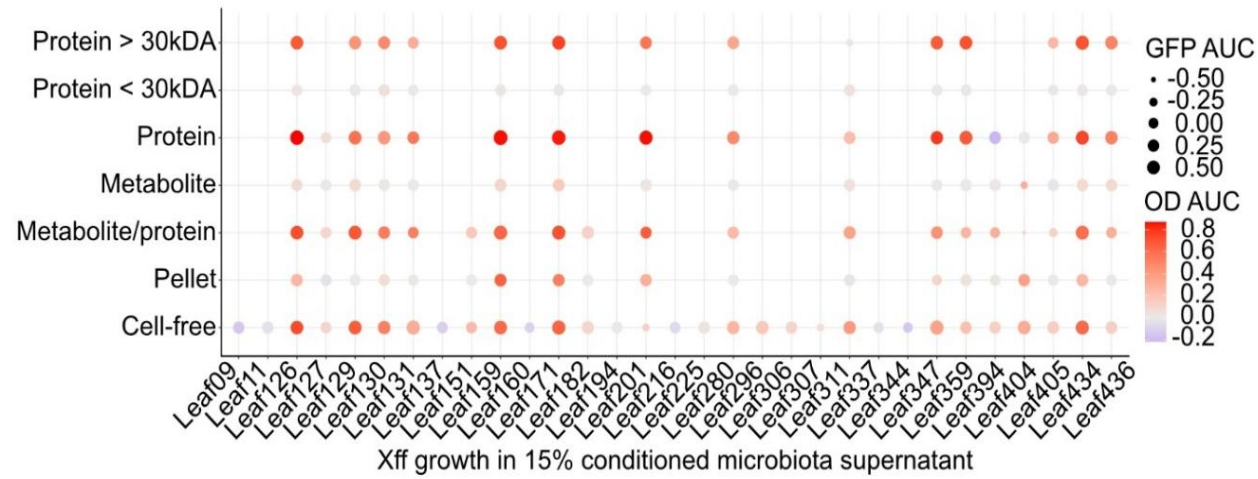


Inter-bacterial interactions

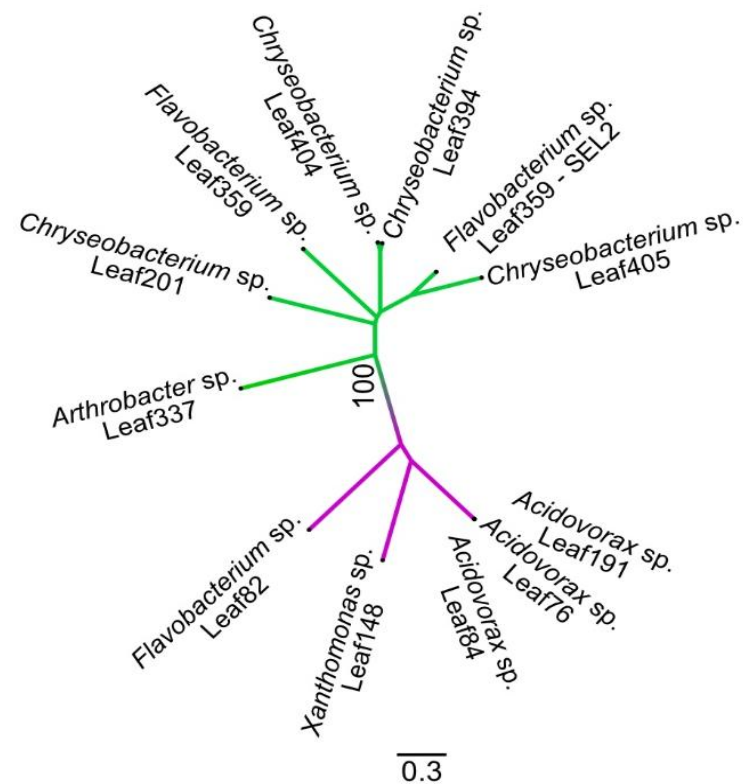
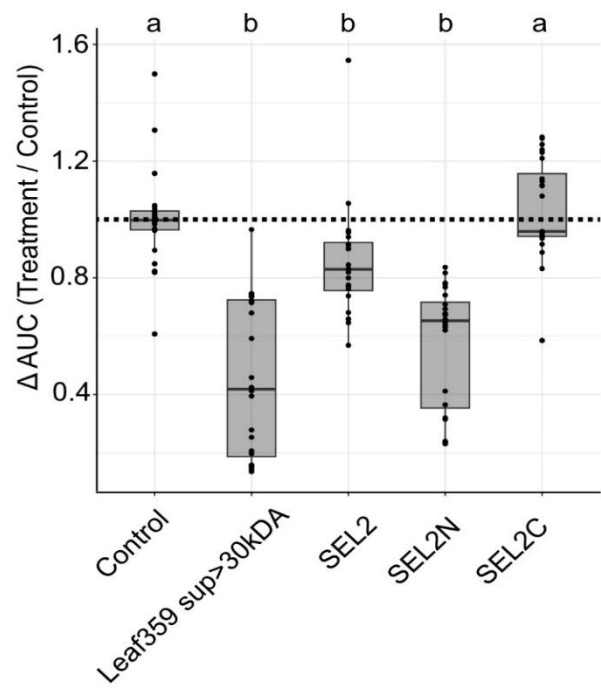
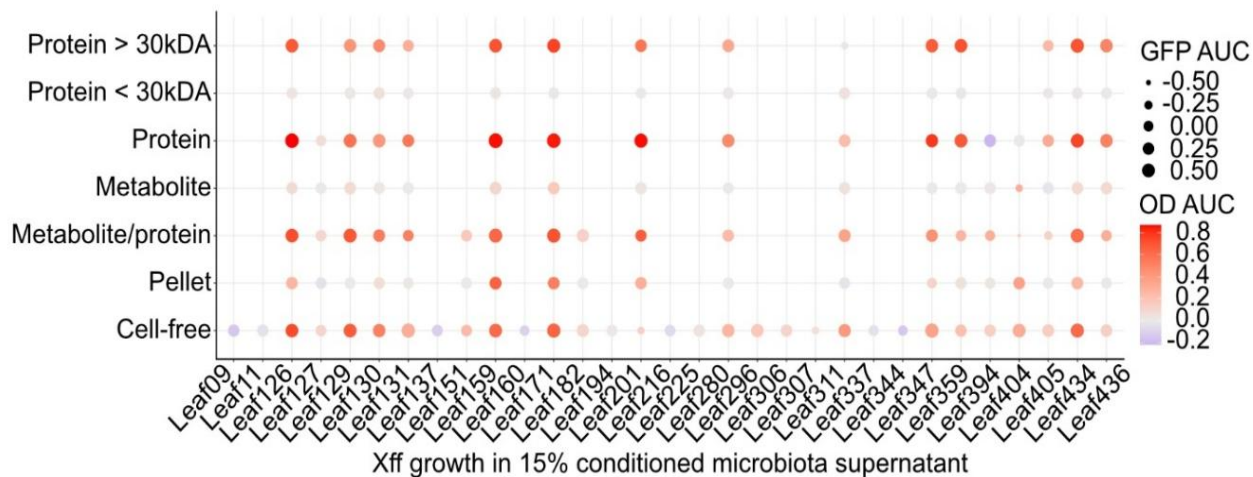
Microbial control of *Xylella fastidiosa* through a novel antagonistic protein from the plant phyllosphere



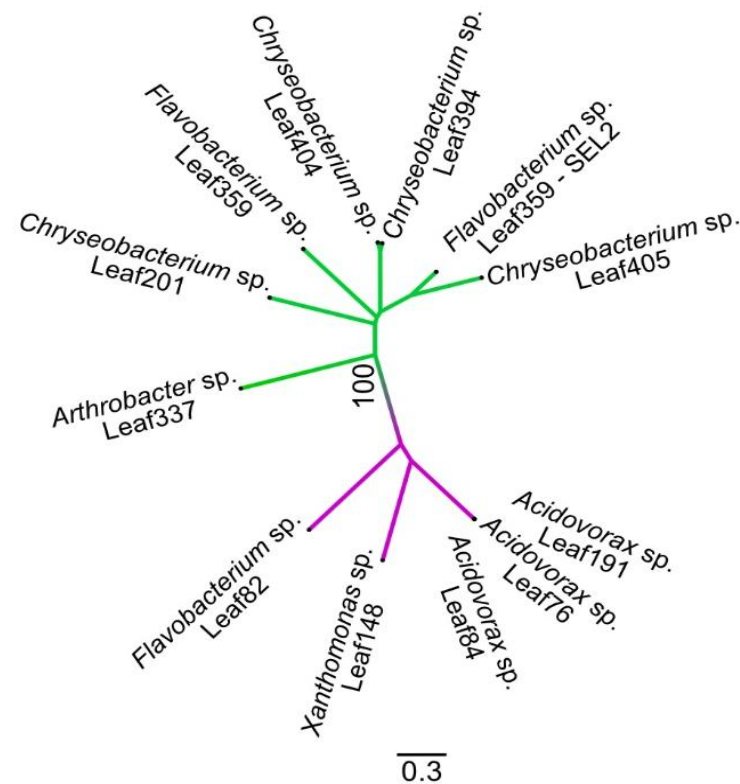
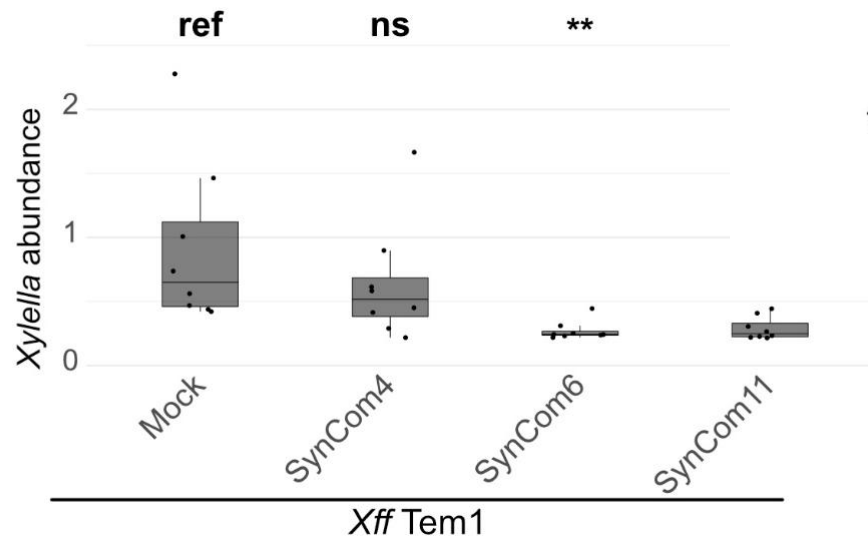
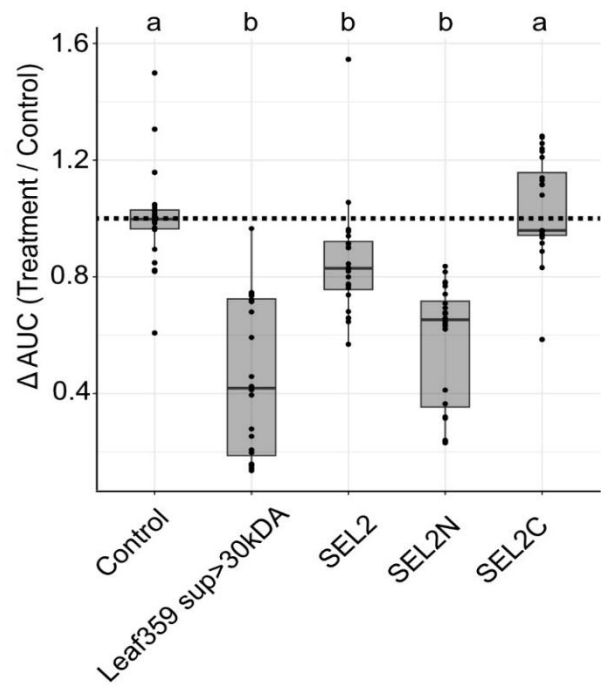
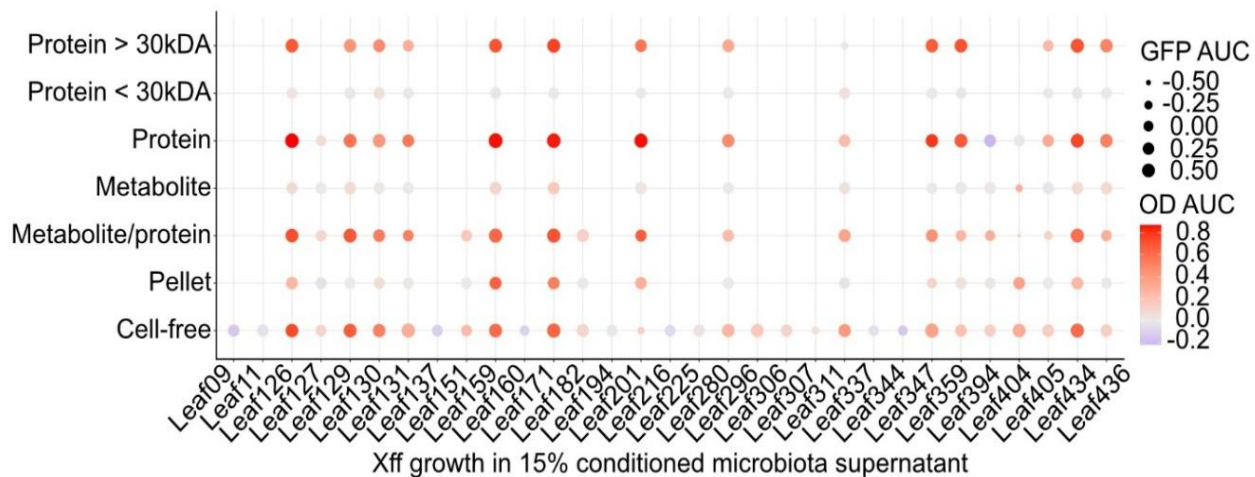
Microbial control of *Xylella fastidiosa* through a novel antagonistic protein from the plant phyllosphere



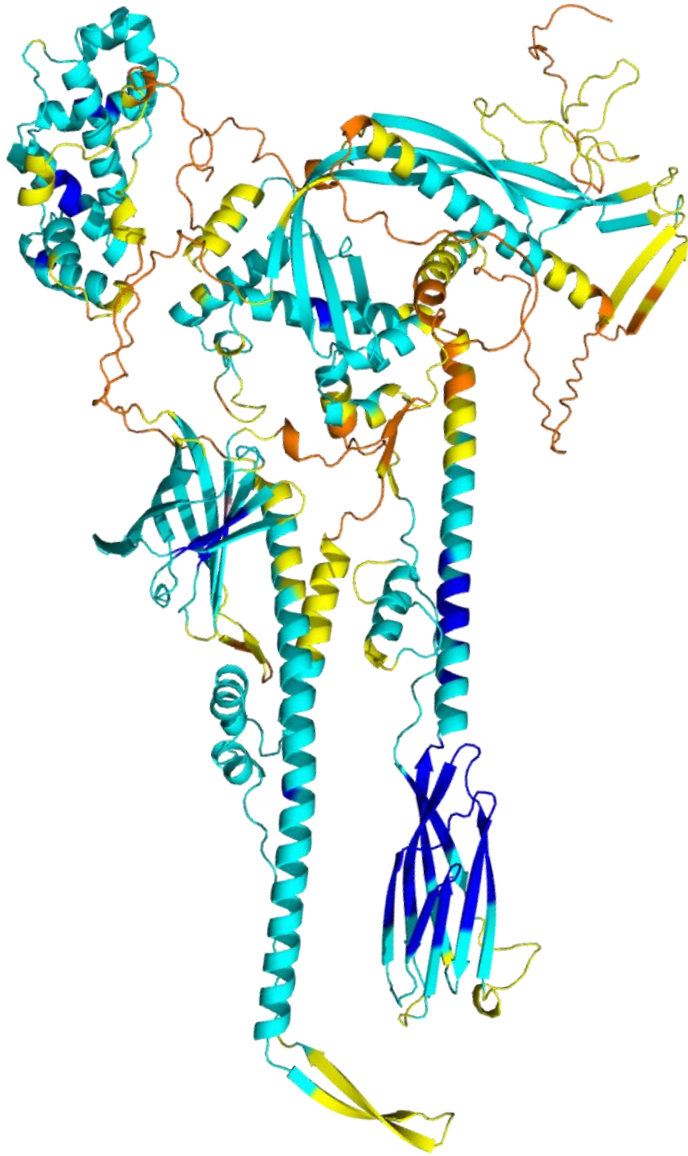
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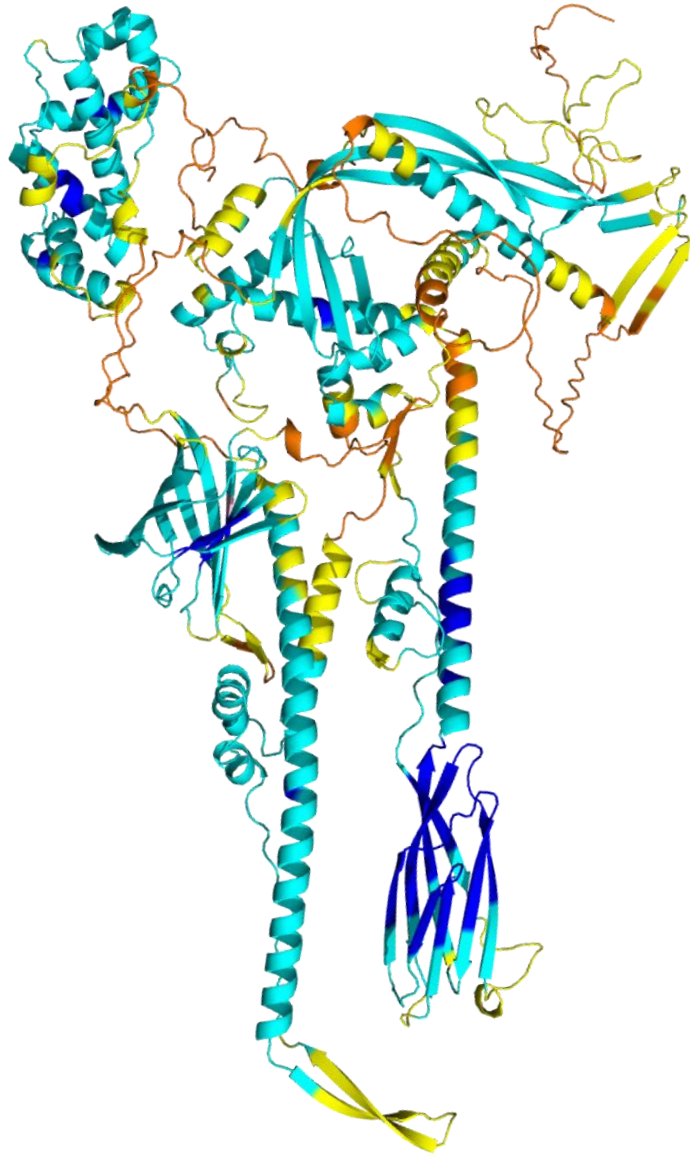
Microbial control of *Xylella fastidiosa* through a novel antagonistic protein from the plant phyllosphere



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



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



**Run to
Poster 68**

Thank You!

AG Robatzek

Jingli Lao

Matias B. Borges

AG Schneeberger

Raúl Wijfjes

Sergio Tusso

AG Parys

Kate Parys



Collaborators

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Pasquale Saldarelli / Maria Saponari (CNR Bari)

Julia Vorholt (ETH Zurich)

Christina Ludwig (TUM)



**Advanced
Grant**
MultiX



Postdoc support fund





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DIPARTIMENTO DI SCIENZE AGRARIE
E FORESTALI



Approcci Nanotecnologici
per un Controllo
Sostenibile e Innovativo
di *Xylella*

IAS  CSIC

NANOTRAPPING *XYLELLA FASTIDIOSA*: MORPHOPHYSIOLOGICAL, MOLECULAR, AND HYPERSPETRAL EVALUATIONS TO UNCOVER MECHANISTIC EFFICACY AND DISEASE REDUCTION POTENTIAL OF CELLULOSE NANOCRYSTAL ENDOTHERAPY

M. A. Muawiya¹, D. Schiavi¹, M. Roman-Ecija², L. Moll², B. B. Landa², G. M. Balestra¹

¹ Department of Agricultural and Forestry Sciences (DAFNE), University of Tuscia, Viterbo, Italy;

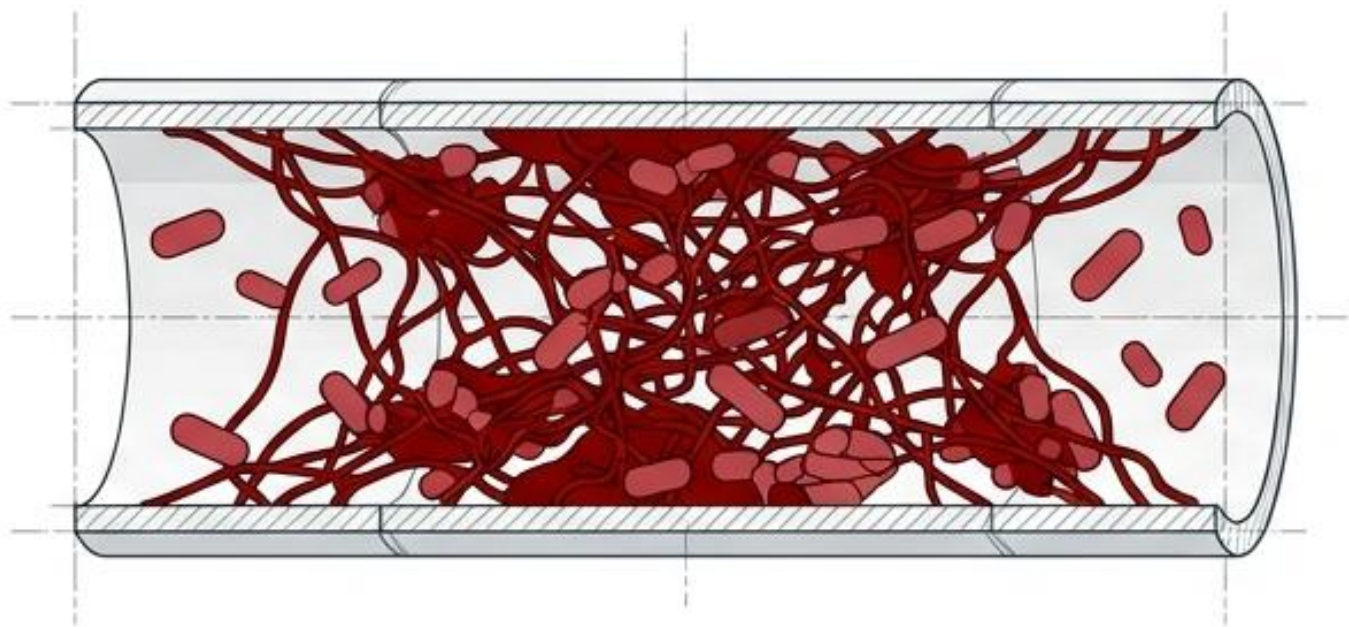
² Institute of Sustainable Agriculture (CSIC), Córdoba, Spain



REGIONE PUGLIA

Mitigating Agricultural Devastation via Sustainable Pathogen Disruption

Xylella fastidiosa threat



X. fastidiosa is a Gram-negative bacterium that devastates global agriculture by forming dense xylem biofilms, lethally disrupting water transport.

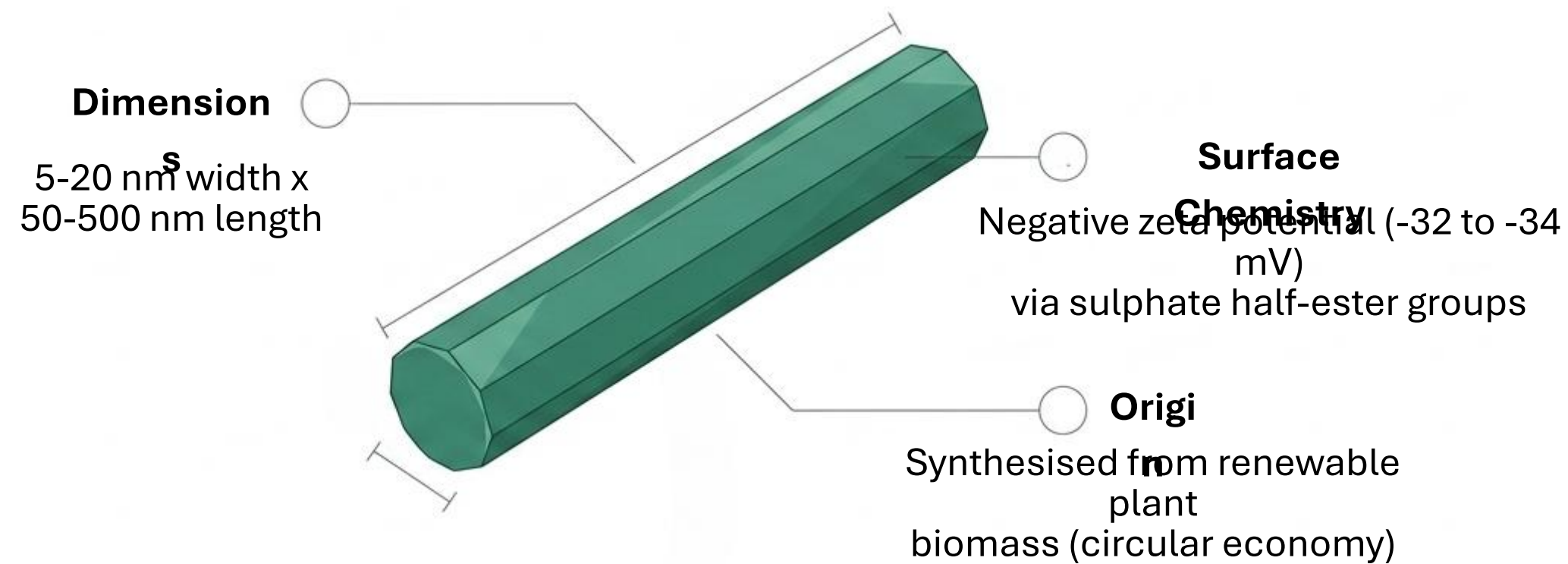
No Curative Treatment Available.

Multi Strain Crisis

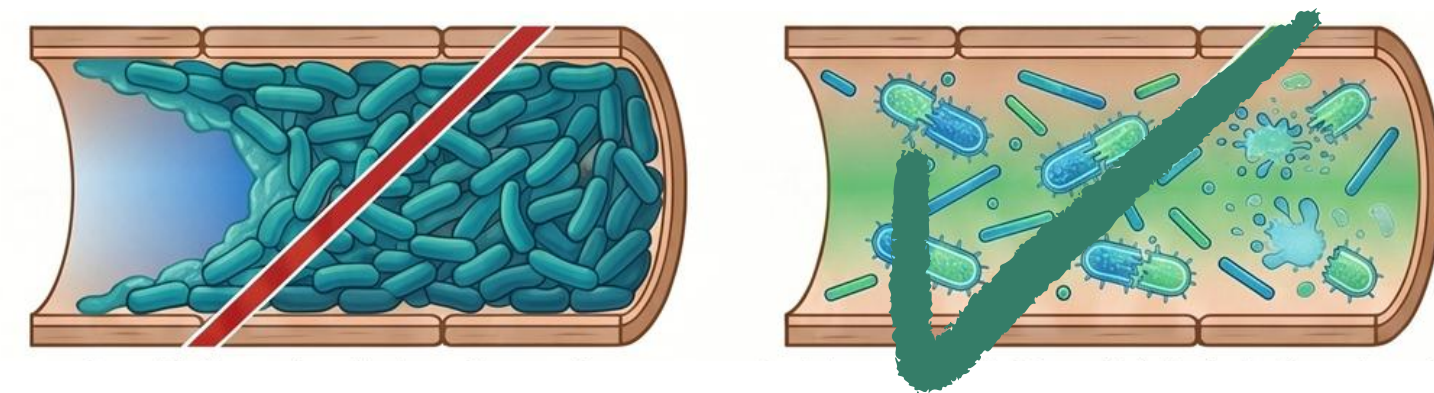
<i>Xylella fastidiosa</i> subsp. <i>pauca</i> • Olive quick decline syndrome (OQDS) • Citrus Variegated Chlorosis (CVC)	<i>Xylella fastidiosa</i> subsp. <i>fastidiosa</i> • Pierce’s disease of grapevine • Almond leaf scorch (ALS))
<i>Xylella fastidiosa</i> subsp. <i>multiplex</i> • Phony peach disease • Plum leaf scald	<i>Xylella fastidiosa</i> subsp. <i>sandyi</i> • Oleander leaf scorch (OLS) • Jacaranda blight/scorch

Our Solution: A biodegradable, target-specific plant endotherapy.

The Circular Plant Protection Weapon (The Cellulose Nanocrystal)



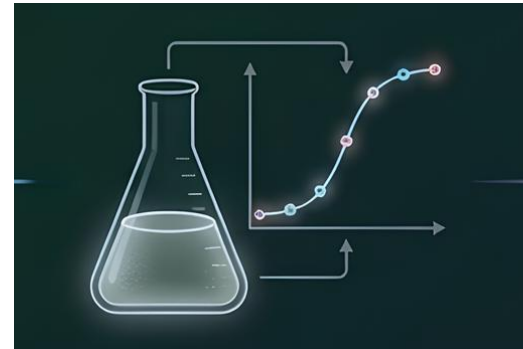
The Physical Sieve Mechanism: CNC execute mechanical, contact-mediated membrane piercing rather than relying on intracellular oxidative or chemical stress. They form a physical blockade without triggering chemical toxicity.



Nanotrapping Hypothesis

Targeted CNC physically lyses *Xylella* envelopes and reduces biofilm, preventing dormancy.

Experimental Framework: From *In vitro* Kinetics to Systemic Rescue



Population Dynamics

Time-kill Kinetics

Continuous turbidimetric growth monitoring and Gompertz kinetic modeling

Vascular Translocation

Visual Disease Progression

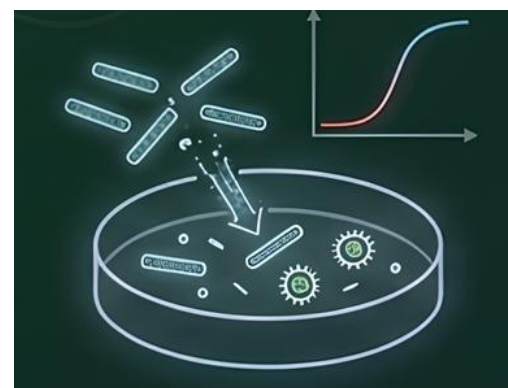
In planta spatial restriction mapping (stem qPCR at 30 dpi).



Nanoscale Interaction

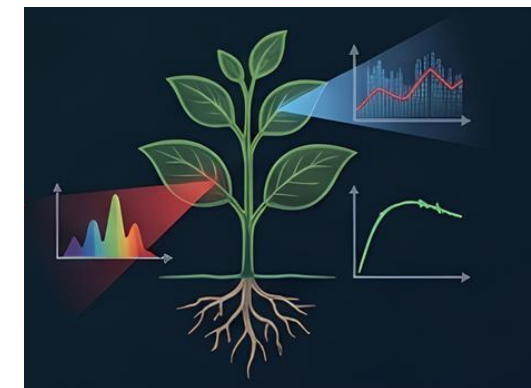
In vitro viability and membrane integrity (v-qPCR).

Biofilm formation kinetics

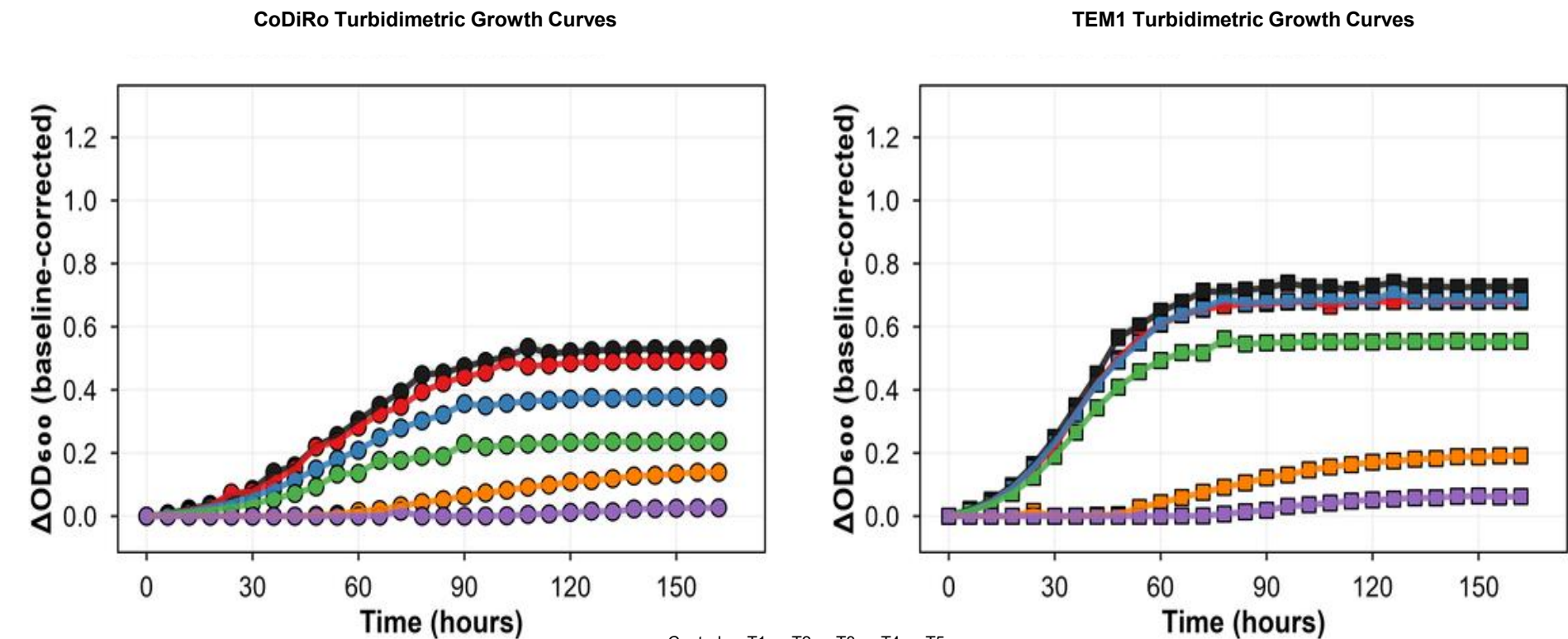
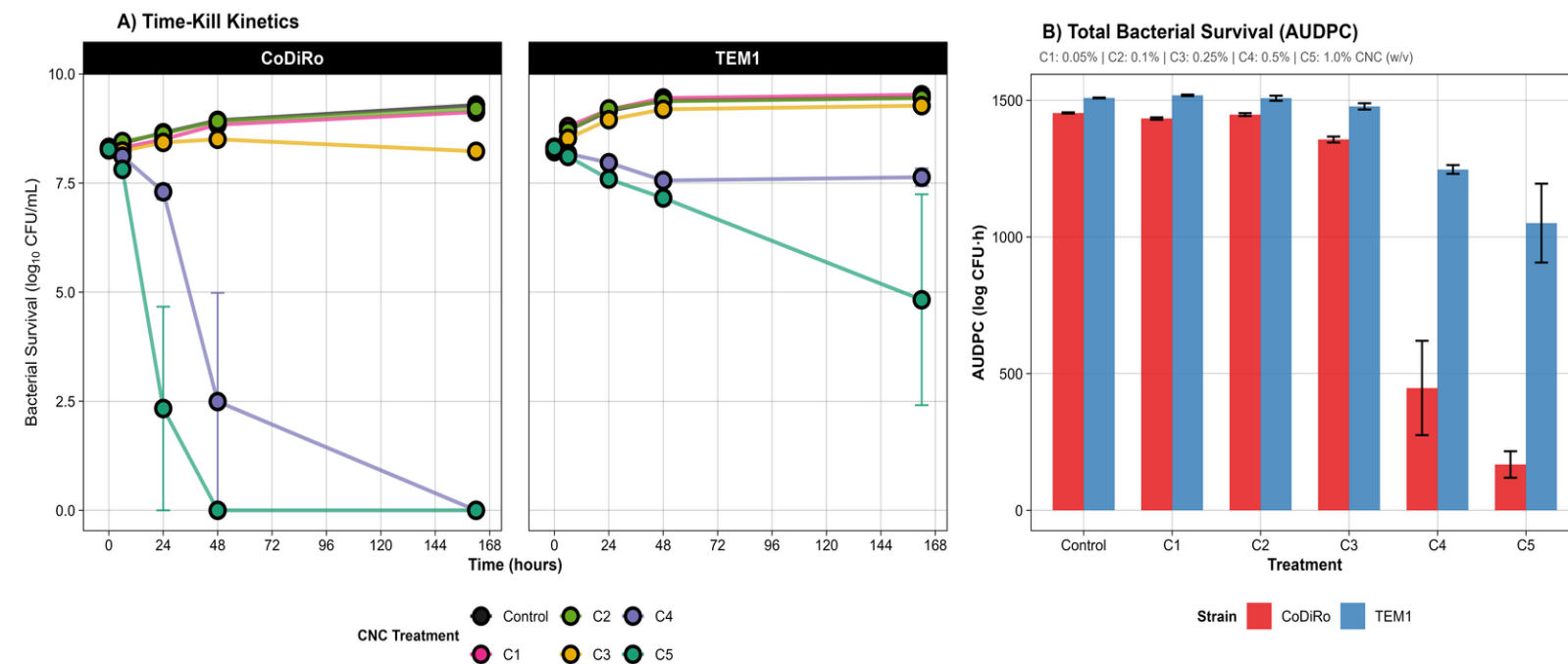


Physiological Homeostasis

Non-destructive whole-plant spectral phenotyping (DualEx/PolyPen at 60 dpi).



Time-Kill Kinetics & Gompertz Modeling: Dose-Dependent Attenuation of Growth



CoDiRo Highlights: Exhibits a sharp, definitive bactericidal response.

- At $\geq 0.5\%$ CNC, populations collapse from 8.30 log 10 to undetectable levels; (< 0.5 log 10 CFU/mL) by 48 hours; > 8.8 log₁₀ unit reduction.

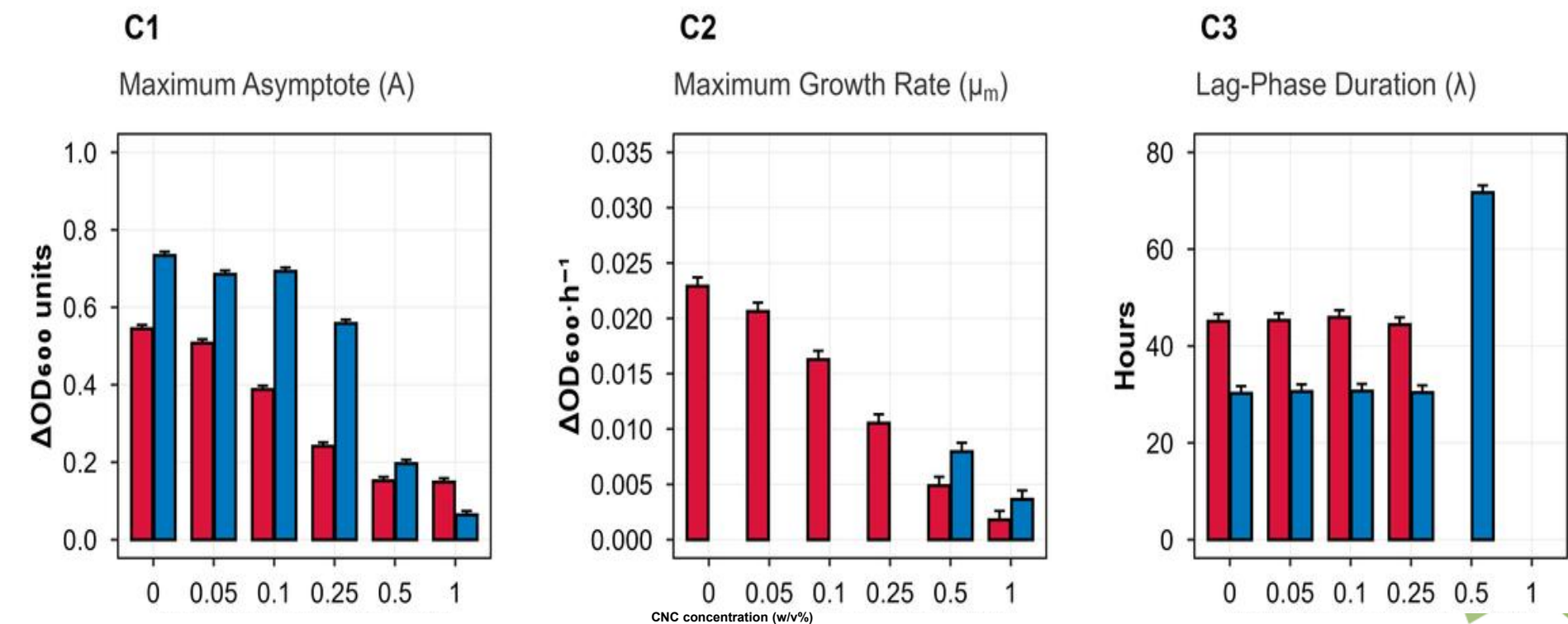
TEM1 Highlights:

- Exhibits robust tolerance and persistence; At 0.5% CNC, maintains viable populations of 7.63 log 10 CFU/mL; At maximum 1.0% dose, a persistent sub-population survives (4.83 log₁₀ CFU/mL).

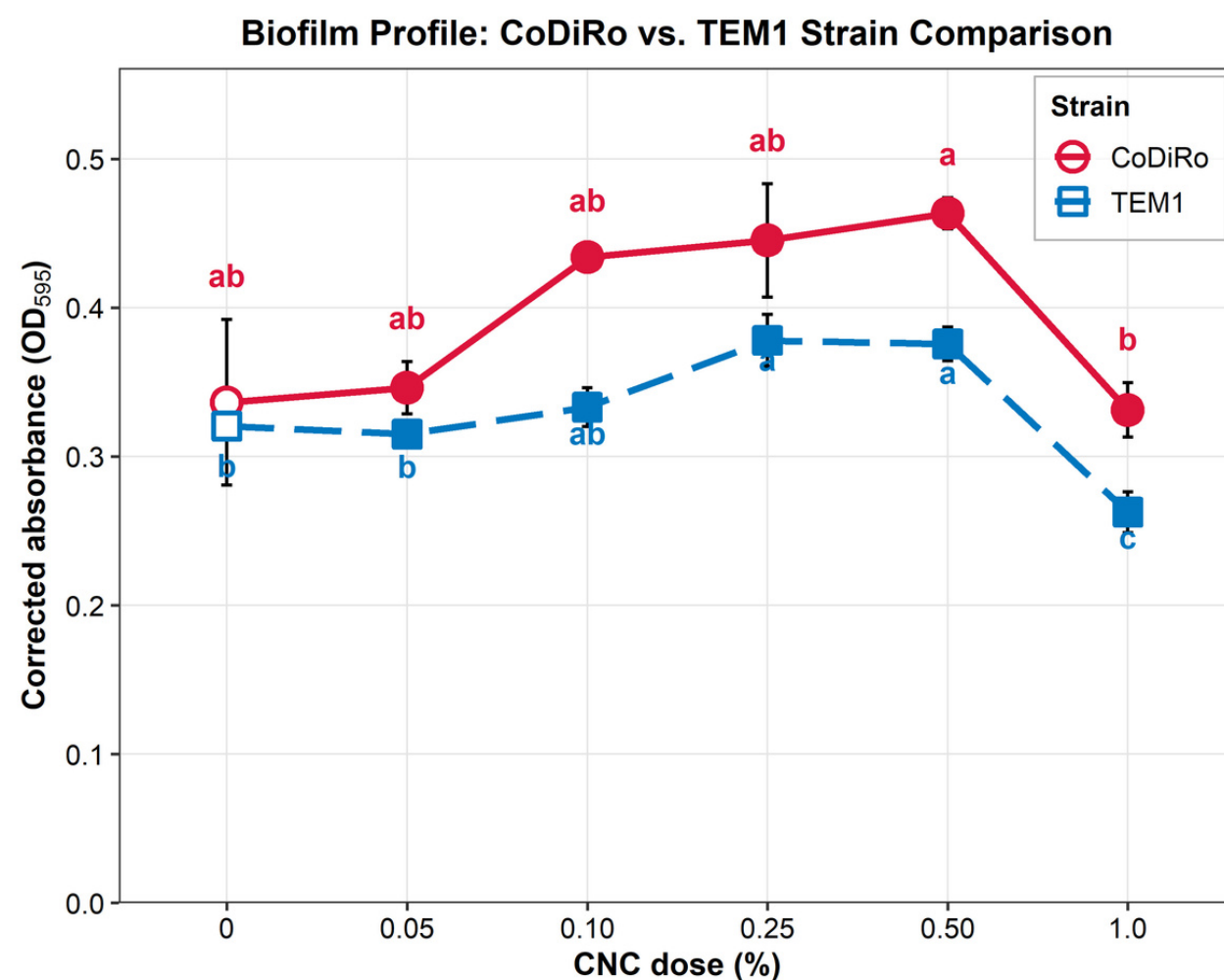
Baseline Divergence: TEM1 naturally exhibits a faster specific growth rate ($\mu_m = 0.0492$) and higher carrying capacity ($A = 0.734$) than CoDiRo ($\mu_m = 0.0229$, $A = 0.545$).

CNC Attenuation: CNC exposure systematically forces a step-wise decline in maximum population asymptote ($p < 0.001$) and specific growth velocity across both strains.

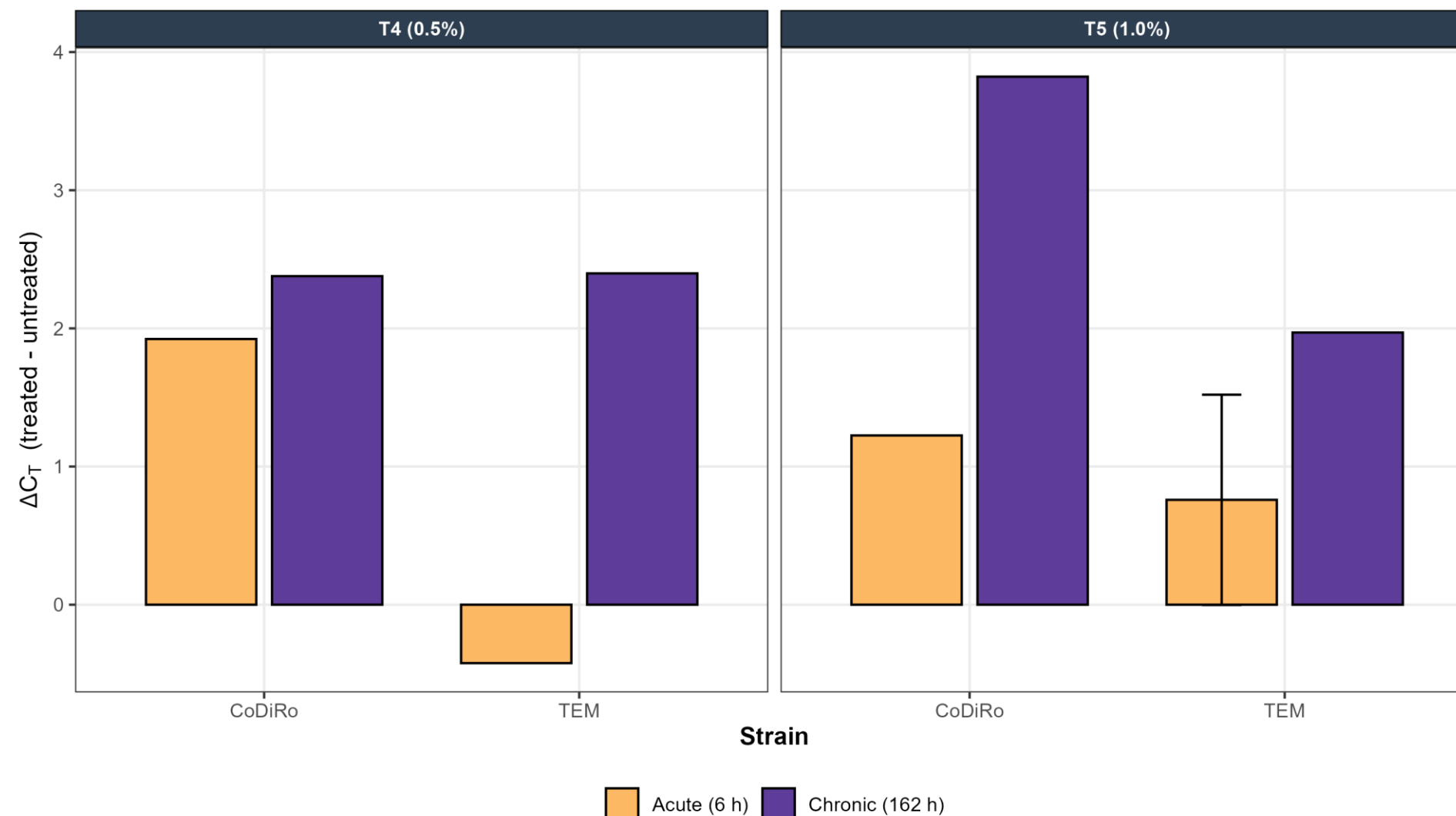
Mathematical Fit: This model ($R^2 \geq 0.994$) confirms a highly predictable, dose-dependent suppression of bacterial fitness.



Biofilm Architecture & Membrane Permeabilization: Hormetic Response & Antibacterial Activity



CNC efficacy: acute vs chronic exposure (high doses)



Reactive Hyper-Secretion and Eventual Collapse:

Rather than immediately dying, bacteria attempt a defensive response at sub-lethal doses.

At the maximal 1.0% CNC dose, this defensive architecture collapses entirely in both strains.

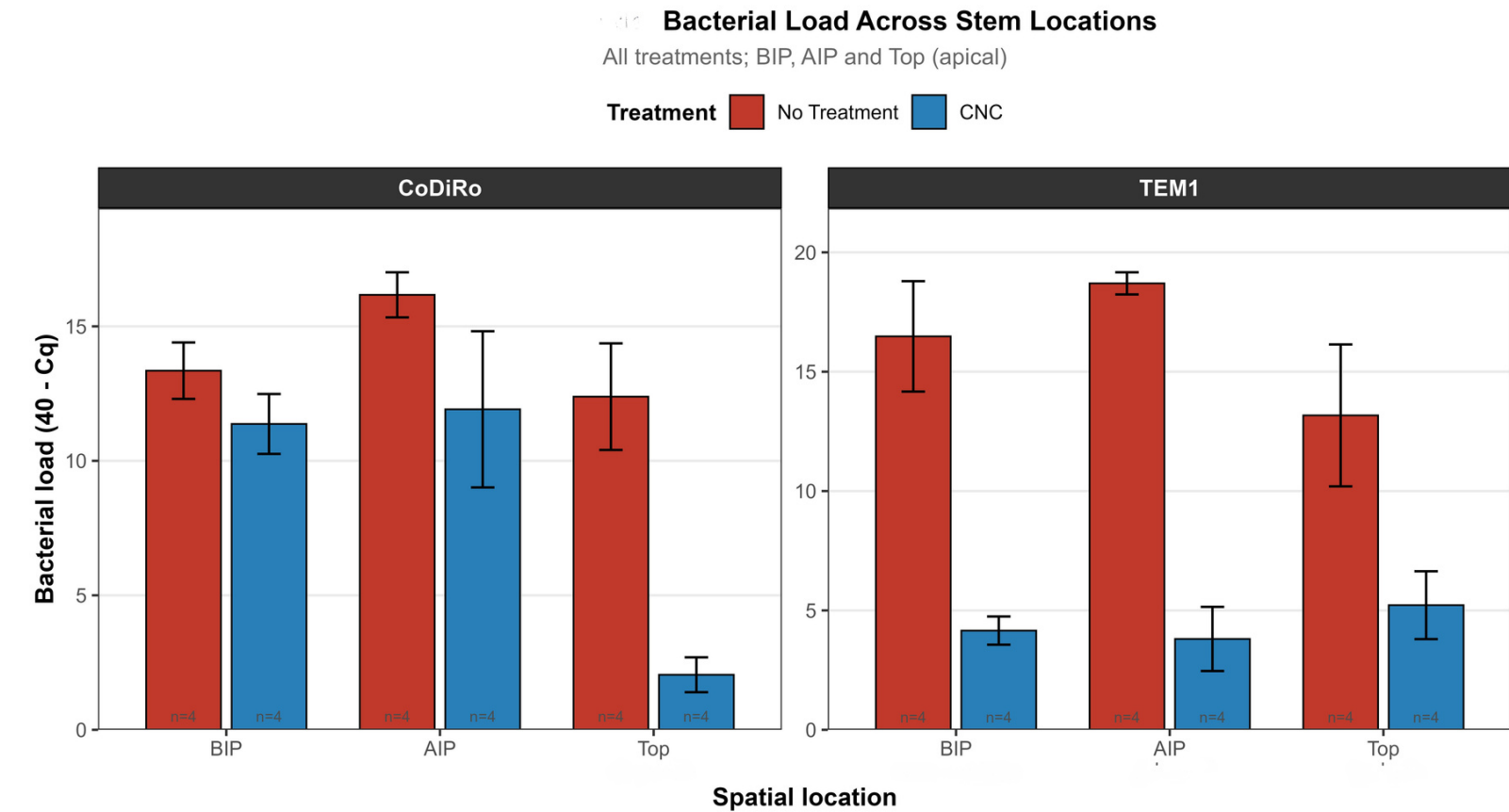
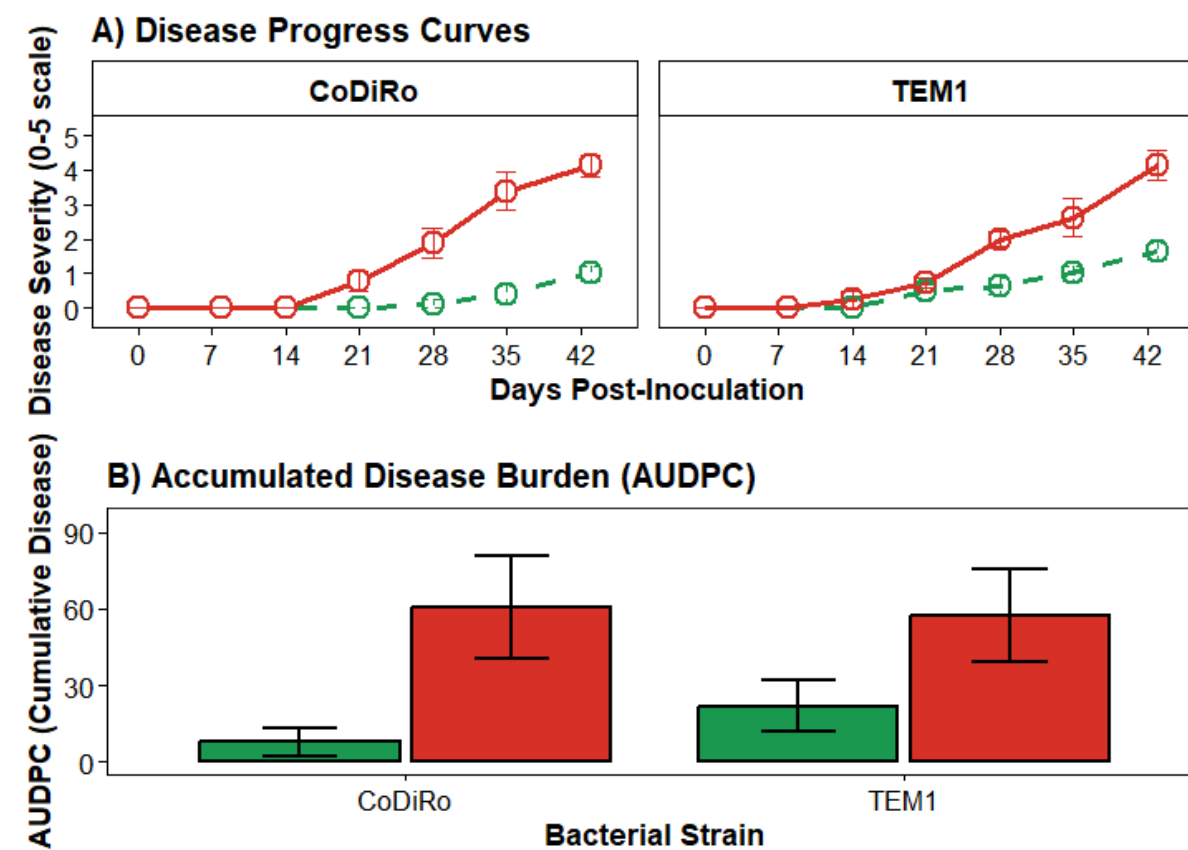
CoDiRo Response:

At 162 hours, high-dose CNC (0.5% - 1.0%) produces massive ACT spikes (1.92-2.38). CNC physically destroys CoDiRo envelopes (Bactericidal).

TEM1 Response:

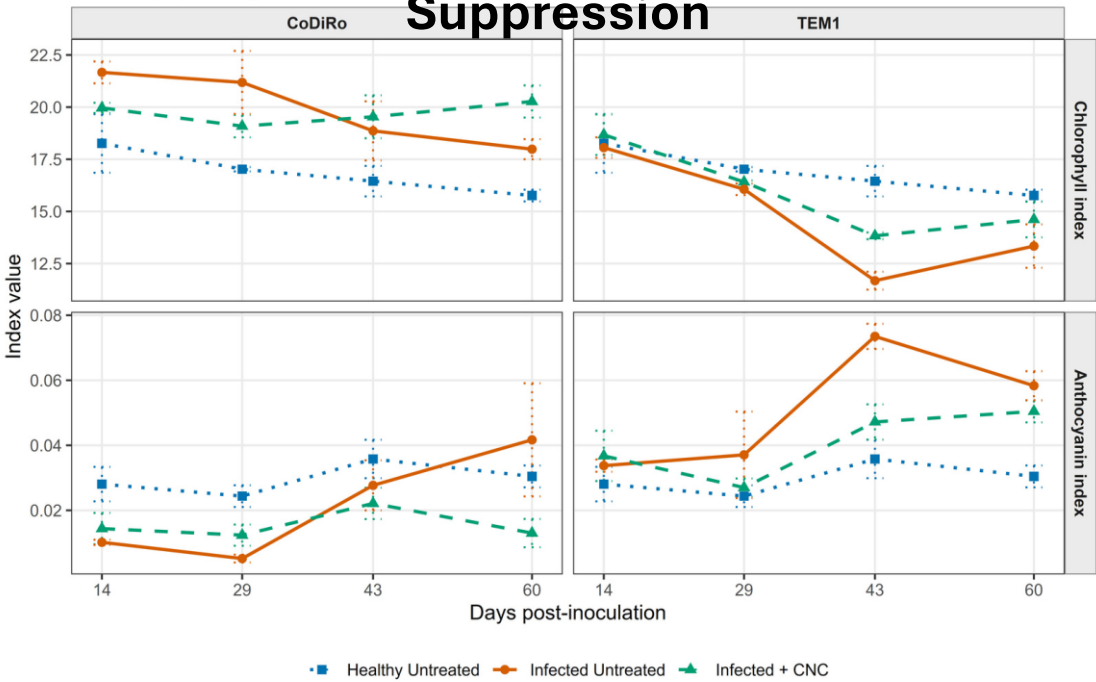
At 162 hours, membrane damage remains significantly lower (<25% compromised fraction). CNC suppresses growth without wholesale membrane destruction (Bacteriostatic).

In planta Evidence: Macroscopic Disease Reduction and Vascular Spatial Restriction



Epidermal Trajectories, Spectral Signatures & Structural Preservation

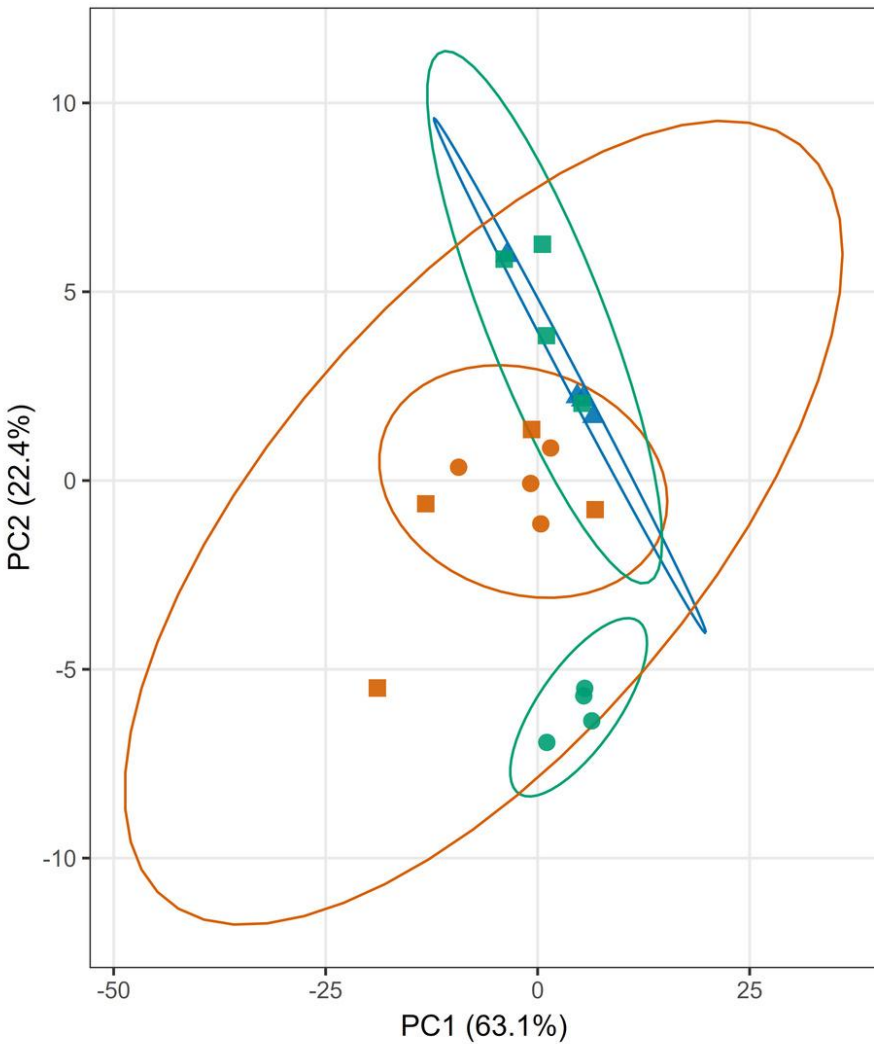
Chlorophyll Rescue & Stress Suppression



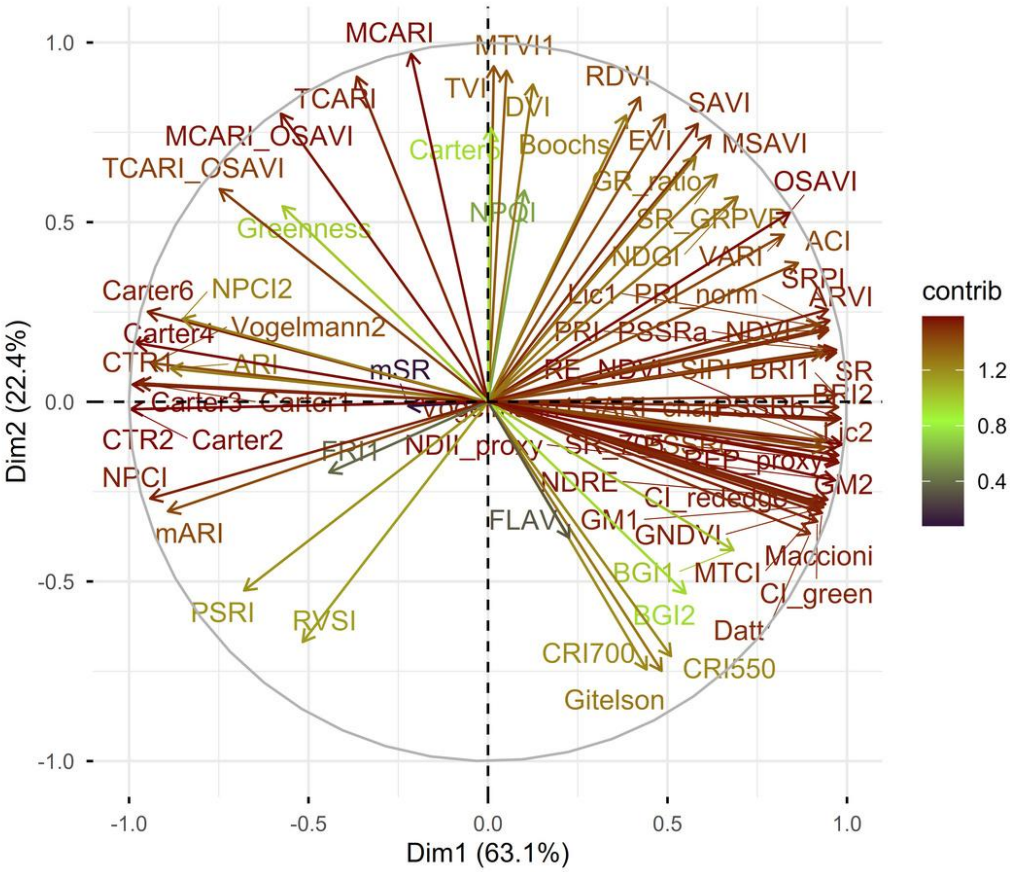
Chlorophyll Rescue: By 60 dpi, CNC treatment hyper-stimulates structural pigment retention (Index = 20.27), significantly exceeding untreated declining plants and even healthy baselines.

Anthocyanin Suppression: Untreated infection triggers a severe secondary-metabolite stress spike (Index = 0.0735). CNC intervention successfully suppresses this systemic stress response, restricting anthocyanin accumulation back to safe levels (0.0472).

PCA of VIs at 60 dpi (95% ellipses)



PCA variable loadings



Multivariate Homeostasis: Return to the Baseline Geometry

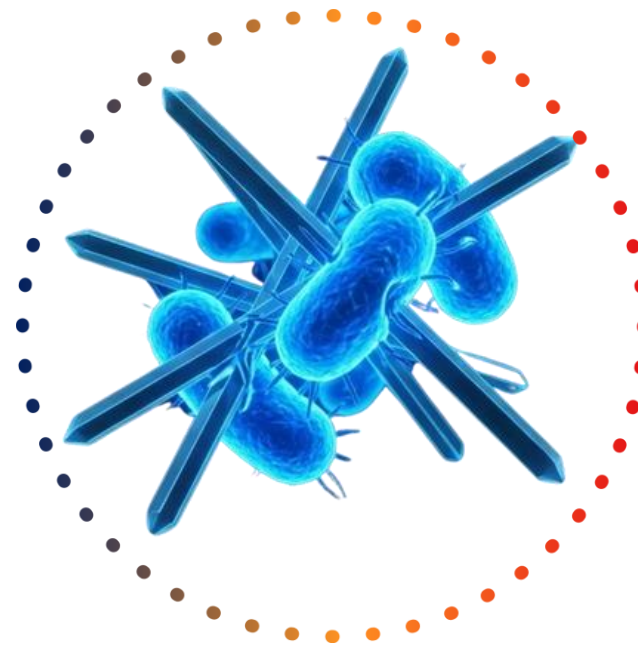
Degraded Optical Space: The untreated diseased plants drift far into a degraded, heterogeneous optical space.

Baseline Recovery: The 1.0% CNC-treated plants strictly co-cluster with the healthy uninfected baseline.

Key Takeaway: CNC-mediated stabilization does not require complete pathogen eradication (as seen in TEM1). It translates partial spatial restriction into total systemic physiological rescue.

The Nanotrapping Mechanism & A Sustainable Future for Vascular Crop Protection

Unlike traditional chemical bactericides (e.g., Copper), CNCs operate via physical entanglement and contact-mediated membrane piercing.



Because they do not induce intracellular chemical or oxidative stress, they do NOT trigger transcriptomic stress pathways (Type II Toxin-Antitoxin systems).

Result: Bacteria are trapped and destabilized without being forced into the highly resilient, unmanageable Viable But Non-Culturable (VBNC) state.



Efficacy

Demonstrates profound strain-dependent suppression, moving from in vitro Gompertz dormancy to 87% in planta disease reduction.



Physiology

Rescues host photosynthetic architecture (NDVI) and prevents systemic anthocyanin stress signaling.



Sustainability

Transforms lignocellulosic agricultural waste into a highly potent, non-toxic, targeted endotherapy.

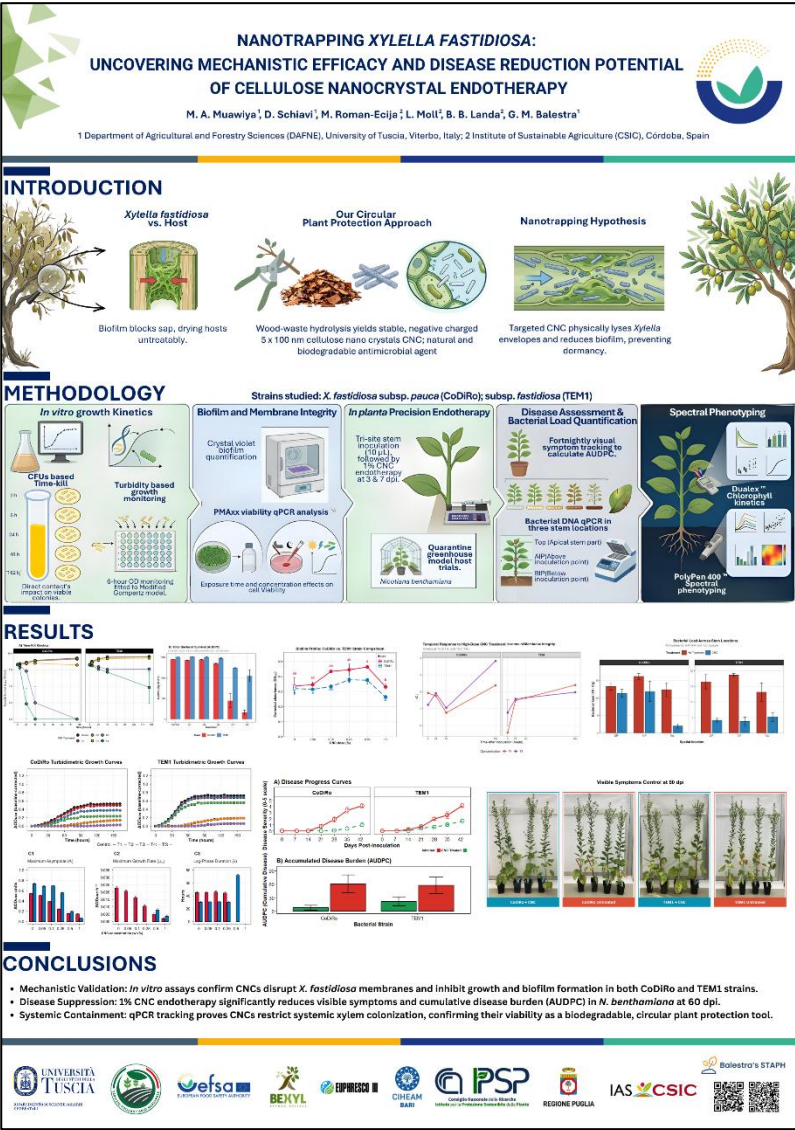
Cellulose Nanocrystals offer a paradigm-shifting, physical biocontrol strategy against quarantine vascular pathogens.

Nanotrapping: A biodegradable endotherapy to overcome *Xylella*.

Thank you!



Poster No. 56



I look forward to sharing more insights and hearing your perspectives at the poster session.