



Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026



REGIONE PUGLIA

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

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(3) Department of Environmental Science, Policy, and Management, University of California Berkeley (UC Berkeley), Berkeley, USA

(4) Corder asbl, Louvain-la-Neuve, Belgium

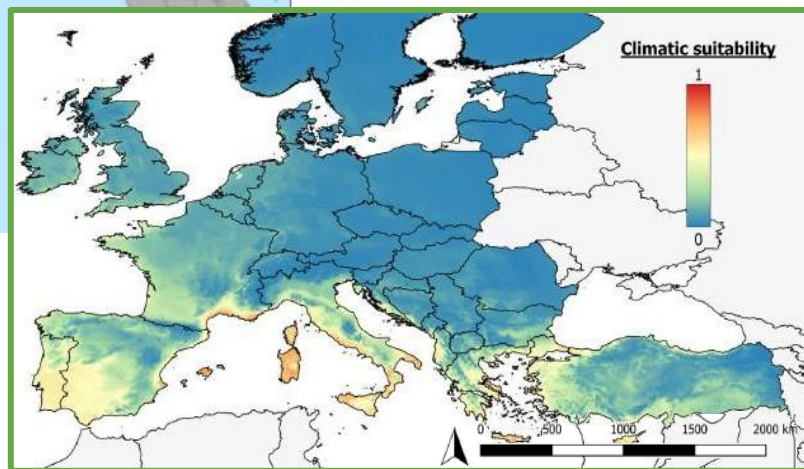


Introduction - Pathogens

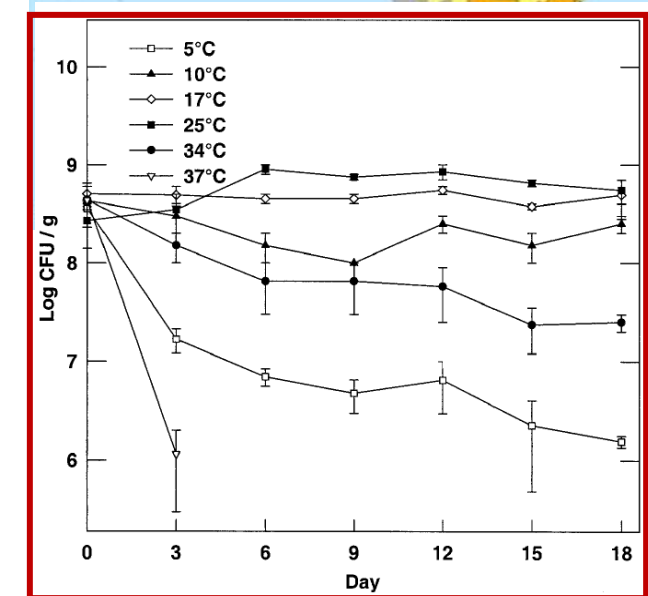
Risk posed by xylem pathogen in Northern Europe should not be underestimated

X. fastidiosa

Plant host and symptoms



(EFSA PLH, 2019)



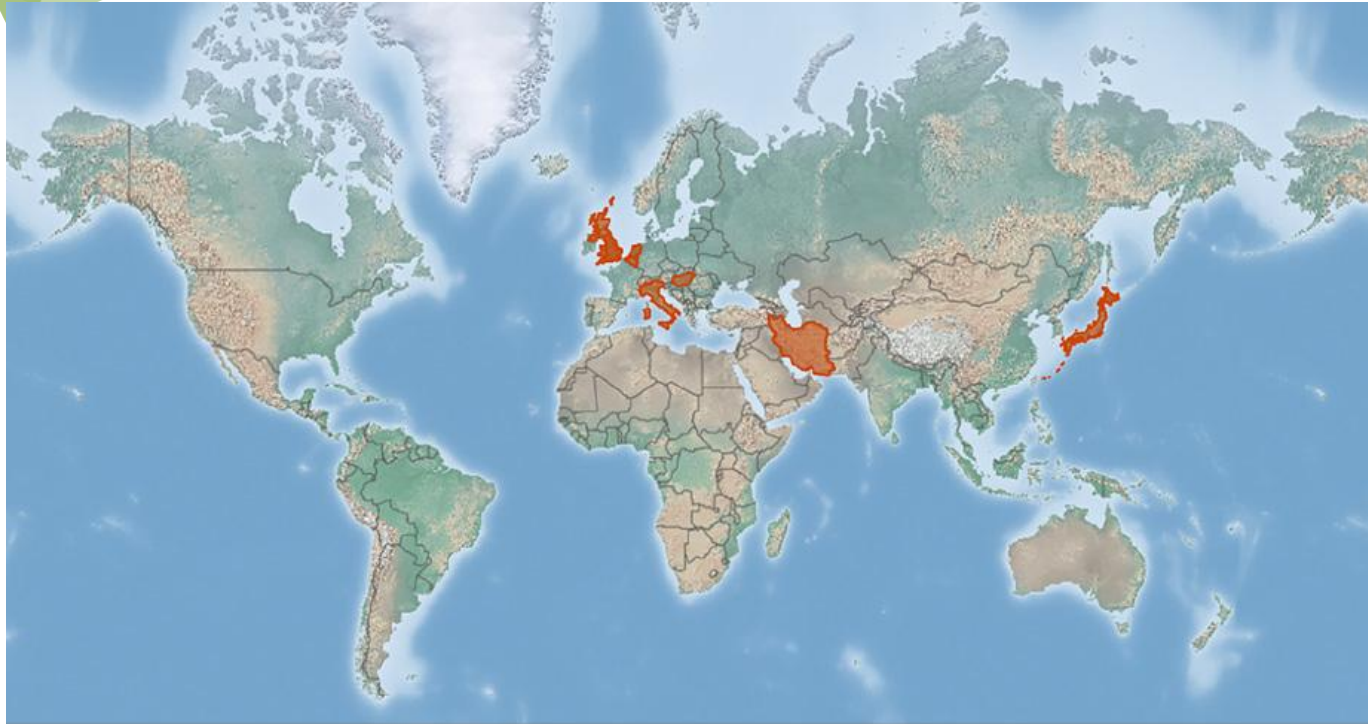
(Feil & Purcell, 2001)

Pesenti, 2025,
CABI Plant Health Case



Risk posed by xylem pathogen in Northern Europe should not be underestimated

B. salicis



CABI, 2024. Brenneria salicis. In: CABI Compendium. Wallingford, UK: CAB International.

● CABI Summary Data

Pesenti, 2024,
CABI Plant Health Case



Willow



Introduction – *Salicaceae* as hosts

Salicaceae are efficient hosts for *X. fastidiosa* and *B. salicis*

- Common in riparian tree layer
- Forming a closed-mesh network

- Hosts of xylem-sap feeding insect potential vectors



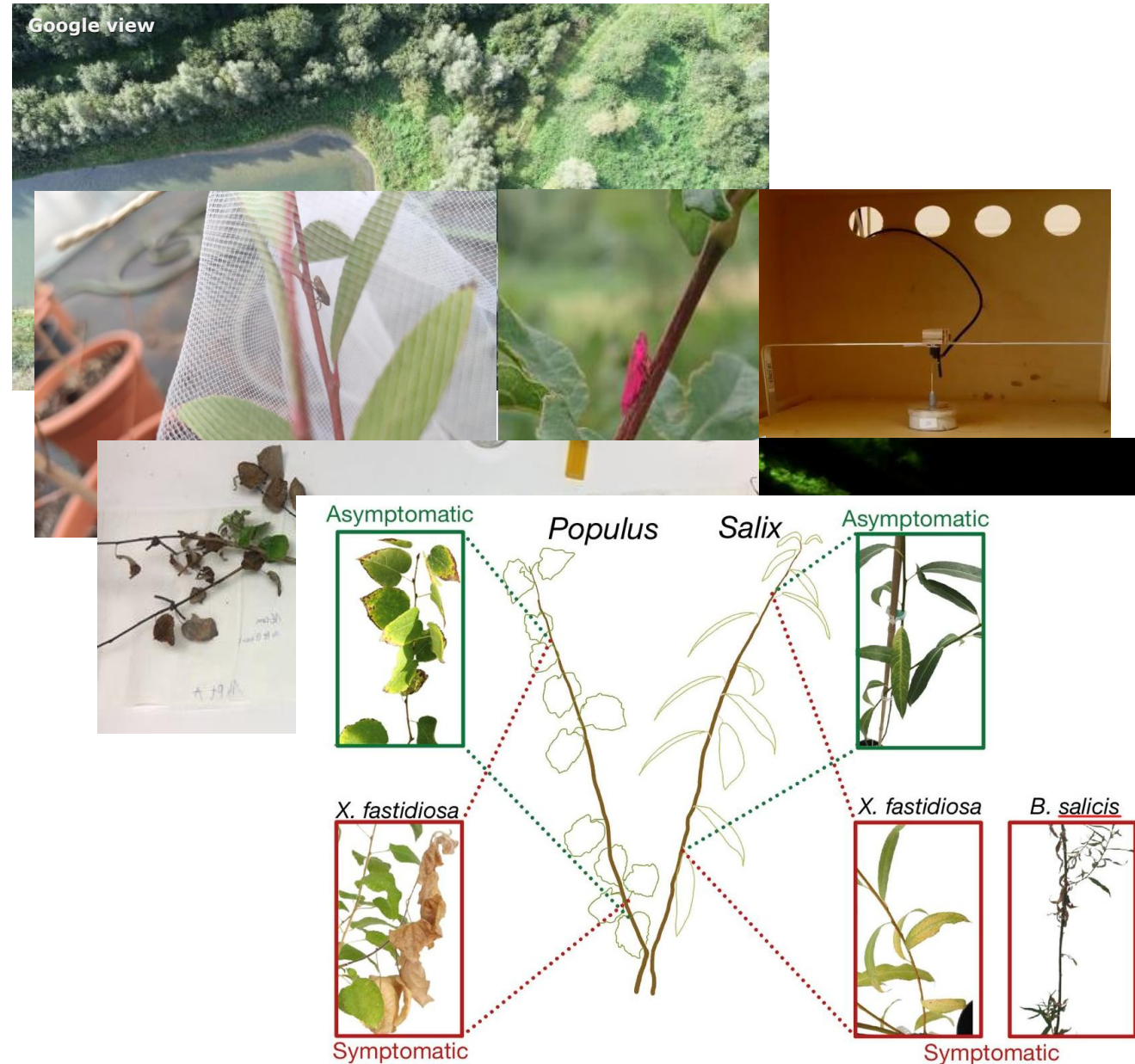
Casarin et al., 2023, J. Pest Sci.

- Multiplication and dispersal in xylem vessels revealed by mechanical inoculation

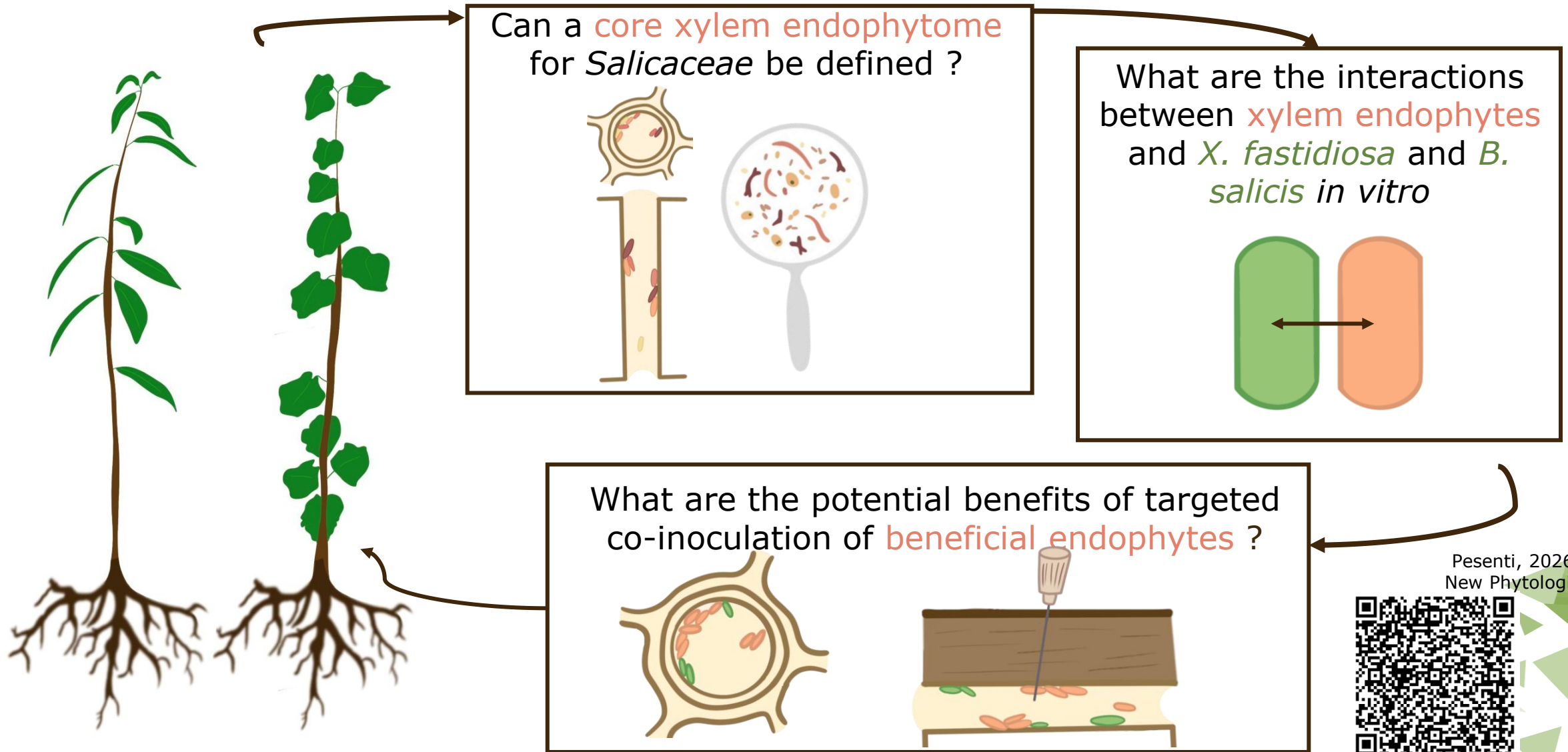


Casarin et al., 2022, Eur. J. Plant Pathol.

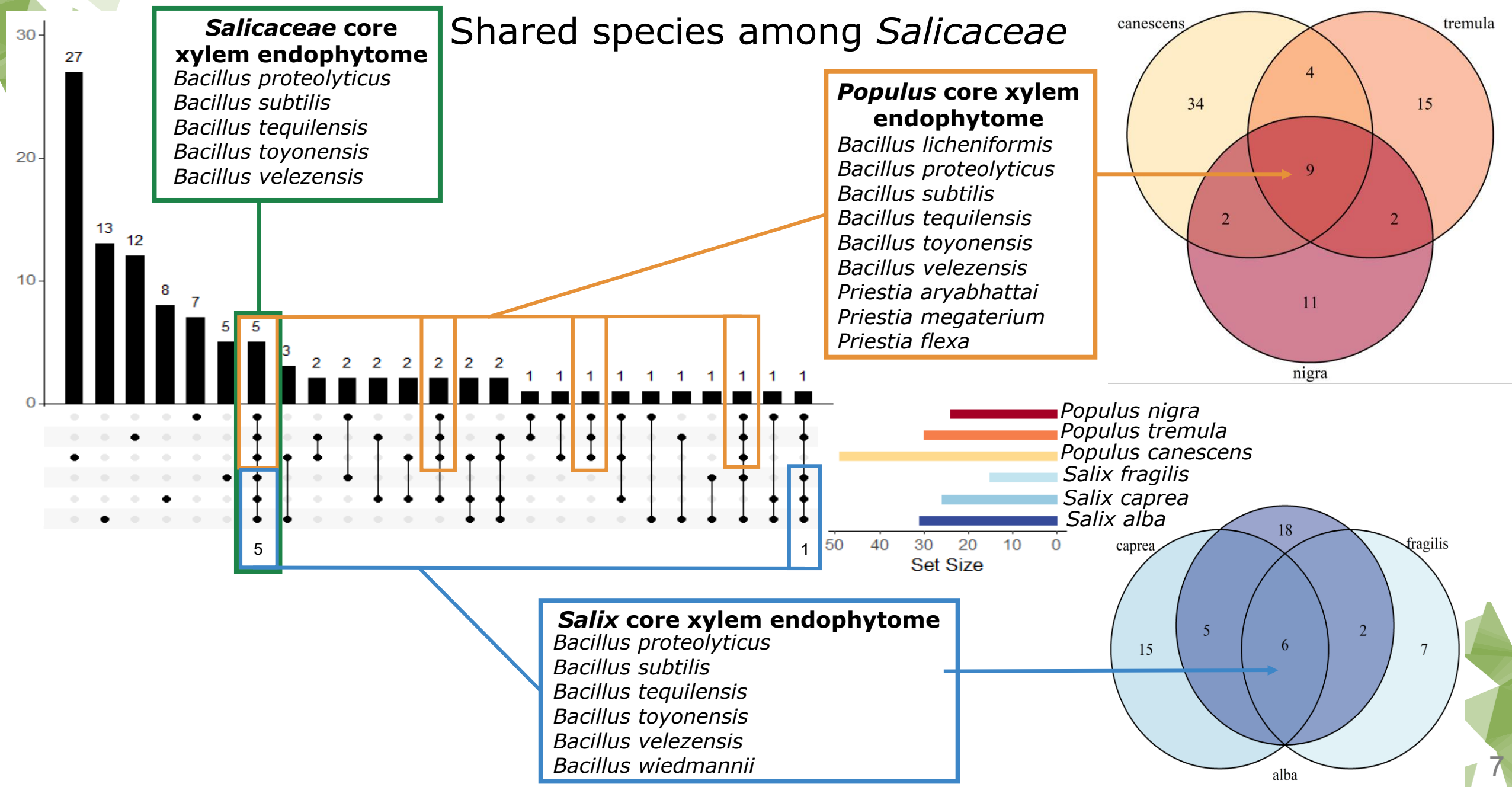
- Plants can be symptomatic or asymptomatic



"Xylem endophytes play a role in mitigating symptom development in host plants infected with *X. fastidiosa* or *B. salicis*"

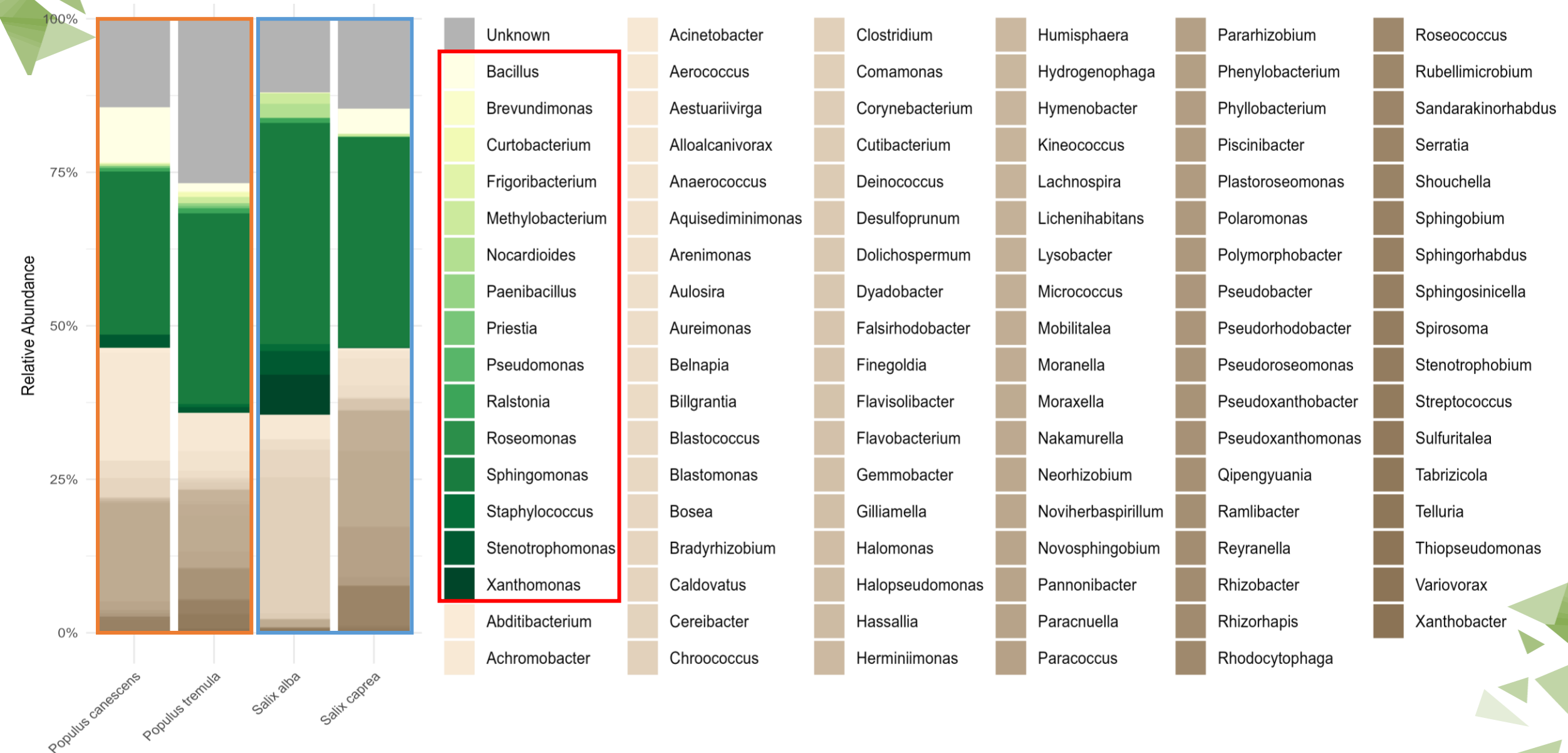


Results - Definition of endophytome via culture-dependent method



Results – Definition of endophytome via culture-independent methods

Relative abundance of each genus (158) in *Populus* and *Salix* samples

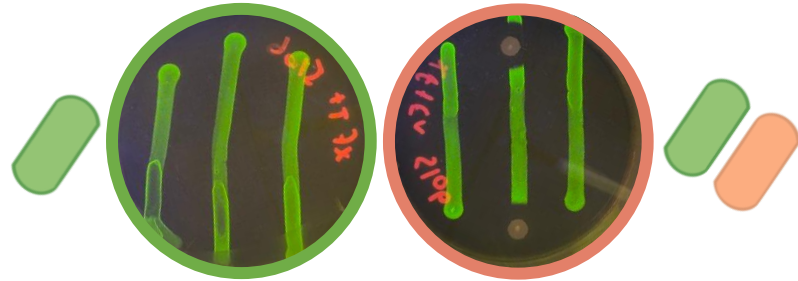


Results – Interaction between isolated endophyte and pathogens

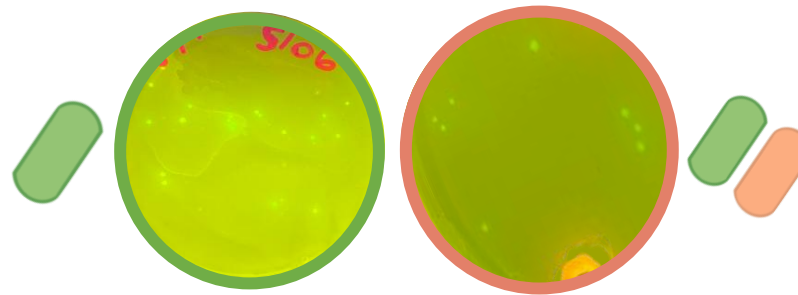
Strains of interest selected based on *in vitro* confrontation and tests

In vitro confrontation

X. fastidiosa

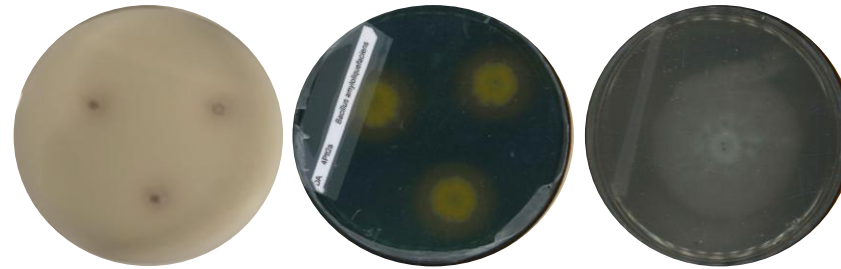


B. salicis

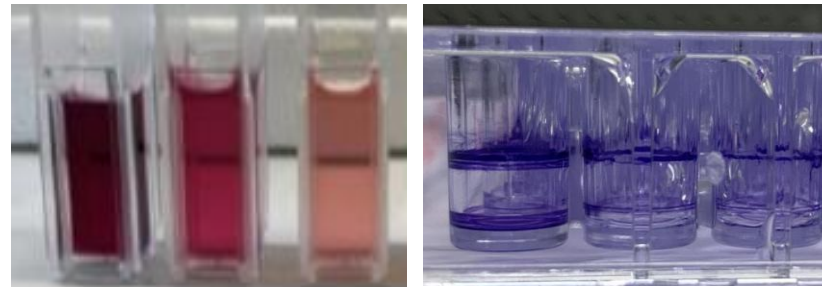


Selection of **40 strains**

Test *in vitro* for beneficial traits

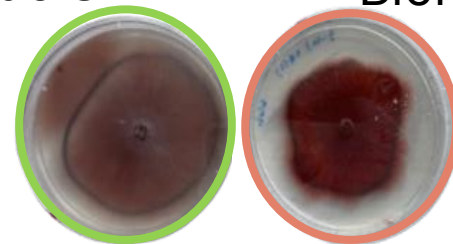


Phosphate Siderophore Motility



Indole

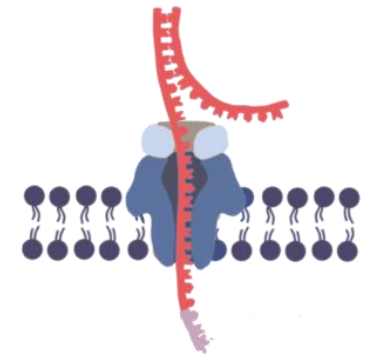
Biofilm



Fungi confrontation

Selection of **15 strains**
based on *in vitro*

Genome & fluorescence



Genome sequencing
& mining



plasmid

Bacterial genome



Transformation

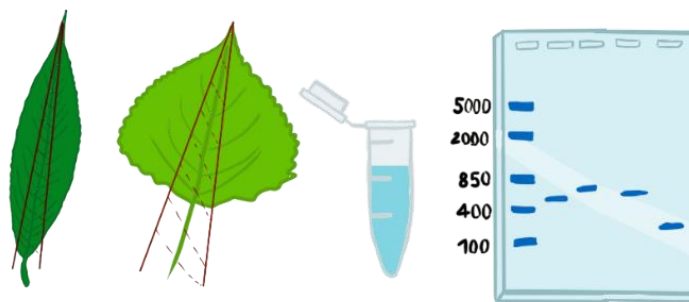
Selection of **5 strains** for *in vivo* inoculation

Methods - *In vivo* inoculation

Symptoms monitoring



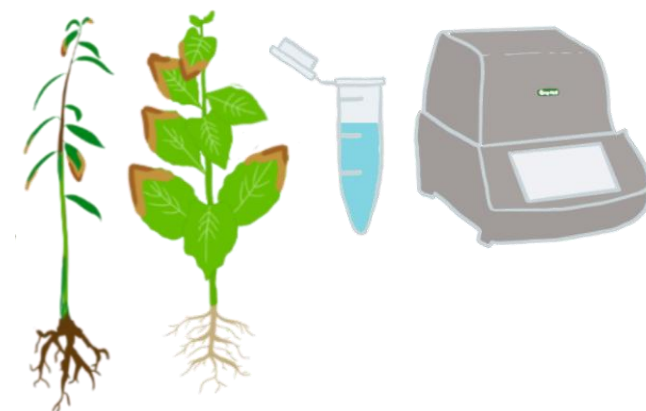
PCR detection



Detection on the leaves

- One-month assay: 2 weeks post inoculation
- Three-month assay: 1 month post inoculation

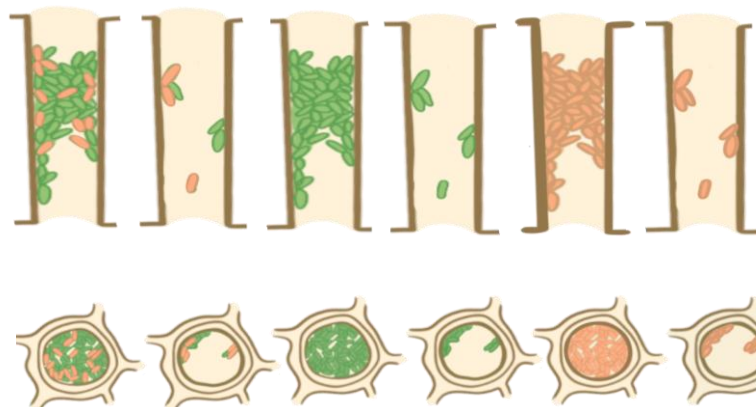
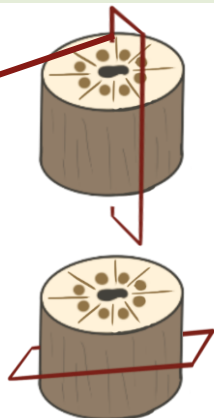
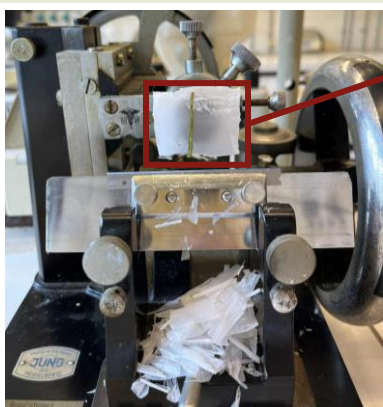
qPCR detection



Detection on the whole plant

- At the end of each assay

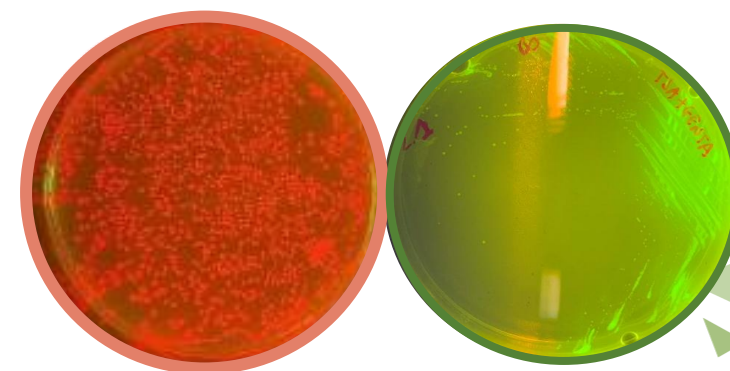
Confocal microscopy detection



Detection on the stems

- At the end of each assay

Re-isolation



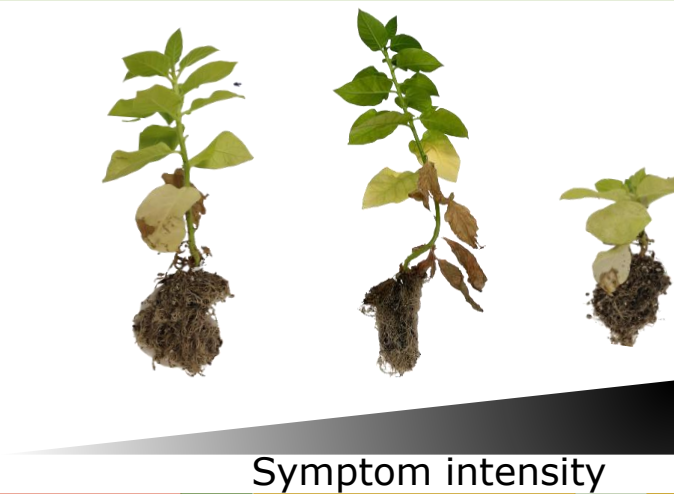
Detection on the stems

- At the end of each assay

Results - *In vivo* co-inoculation

Symptoms monitoring via visual scale and plant height

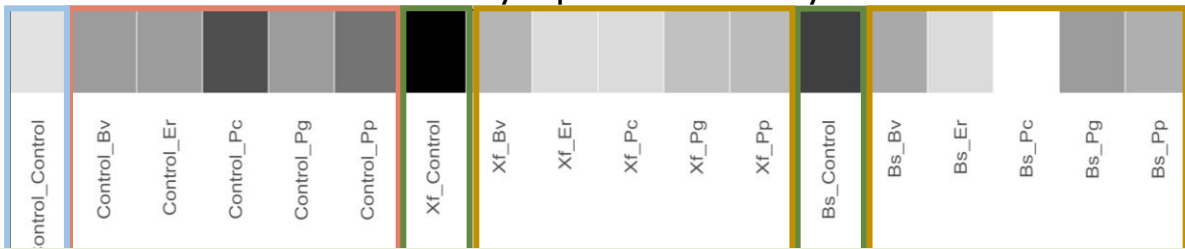
Tobacco assay



Willow assay

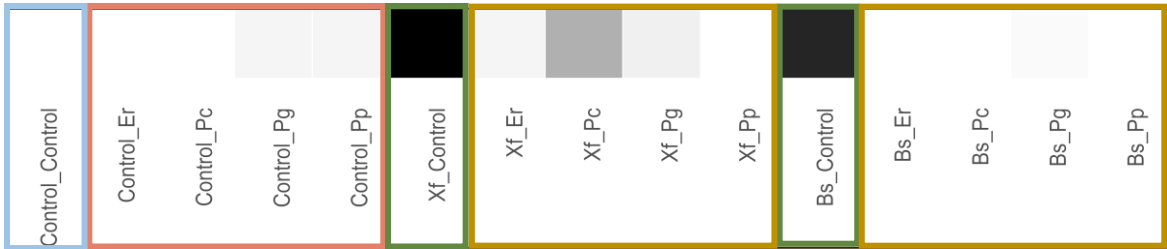


Three-month assay



One-month assay

Mitigation of visual symptom severity in plants co-inoculated with any endophyte across all assays



Three-month assay

Symptoms monitoring via visual scale and plant height

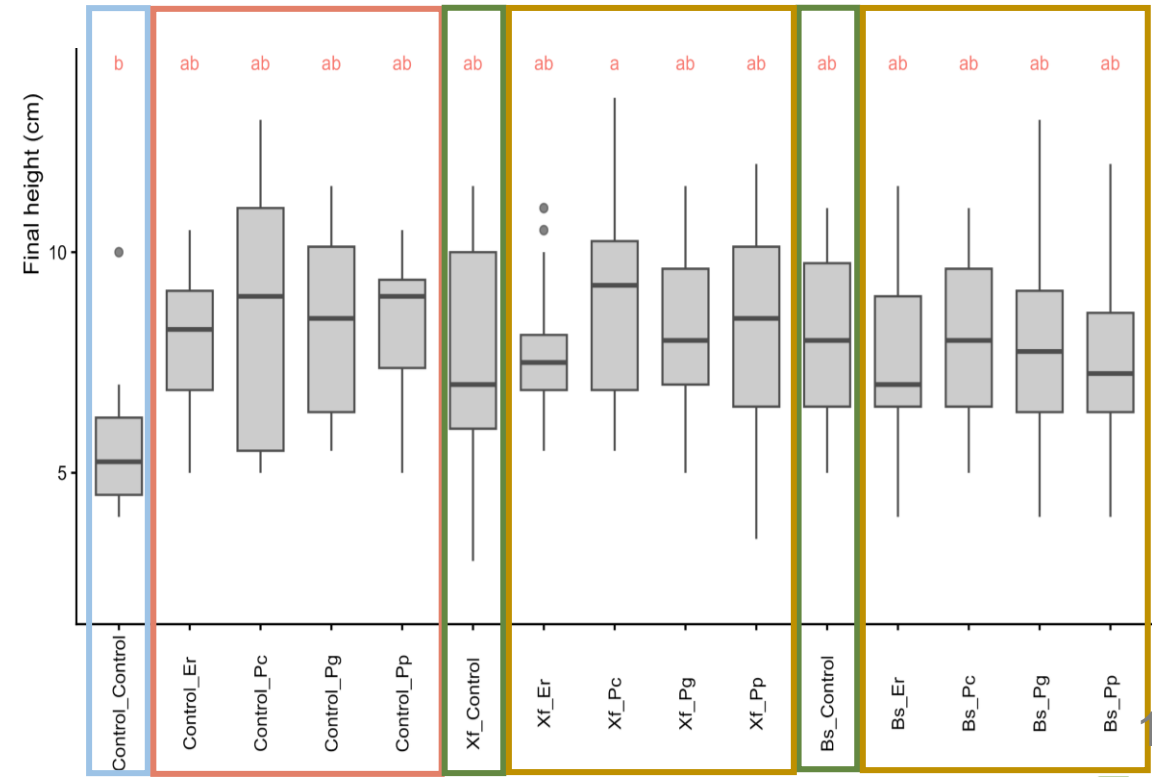
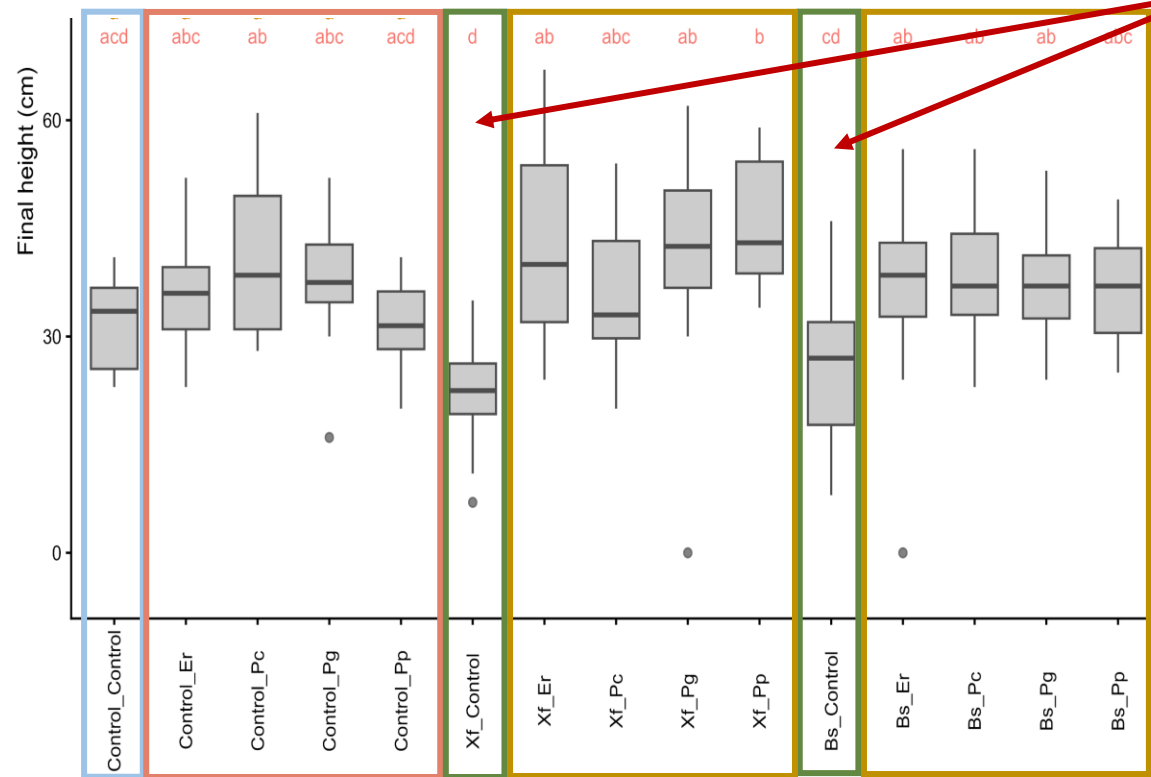
Tobacco assay

Willow assay

No significant difference compared to control plant

No significant difference compared to control plant

Height restauration of co-inoculated plant with any endophyte in tobacco after 3 months

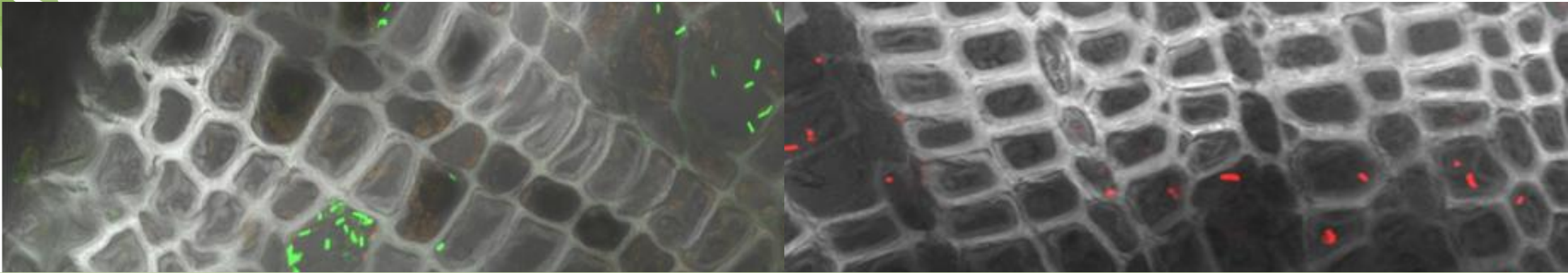


One-month assay

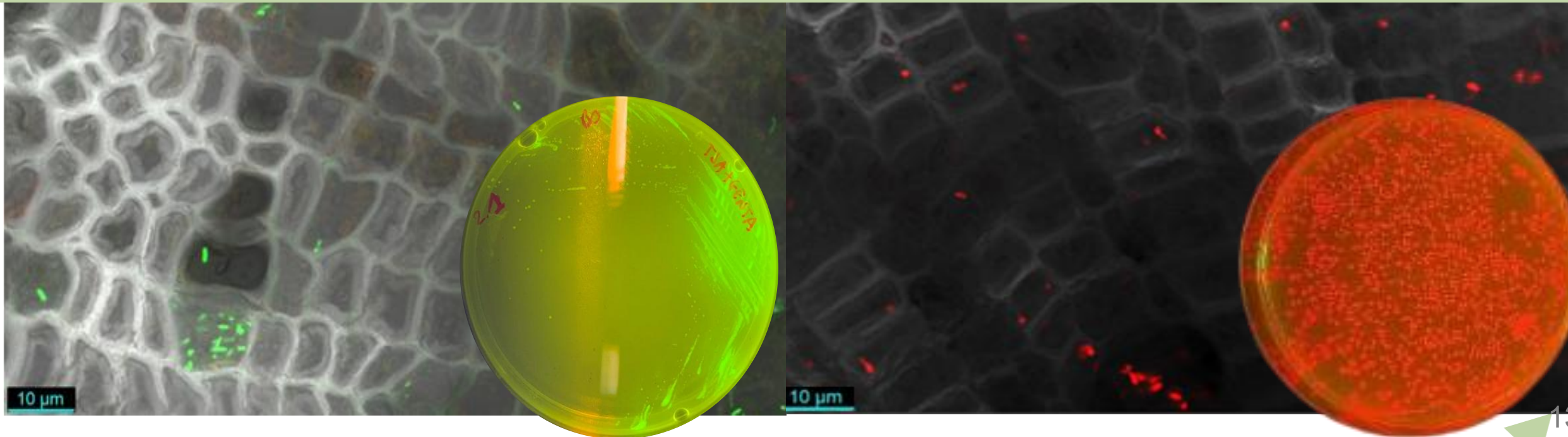
Three-month assay

Results – Pathogen & endophyte visualisation

No co-localization of pathogen and endophyte in the same vessels



Bacterial viability confirmed with re-isolation

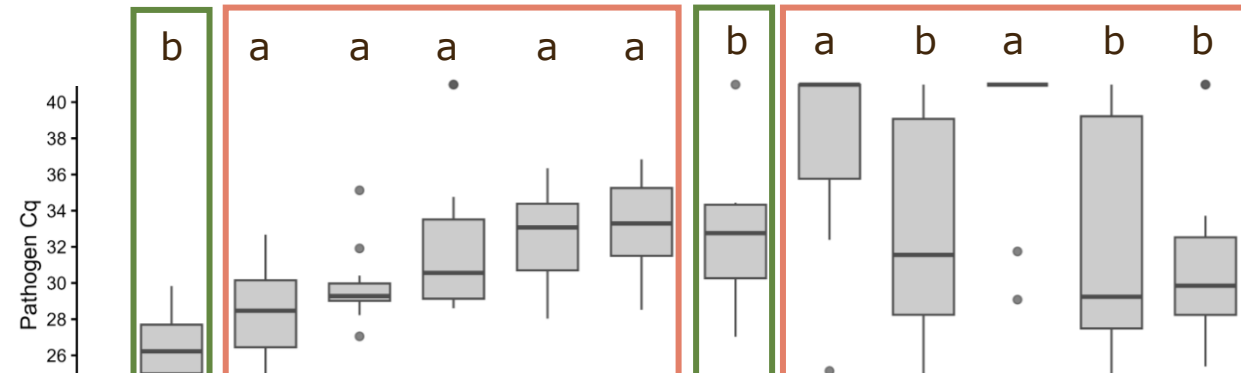




PCR and qPCR detection

Tobacco assay

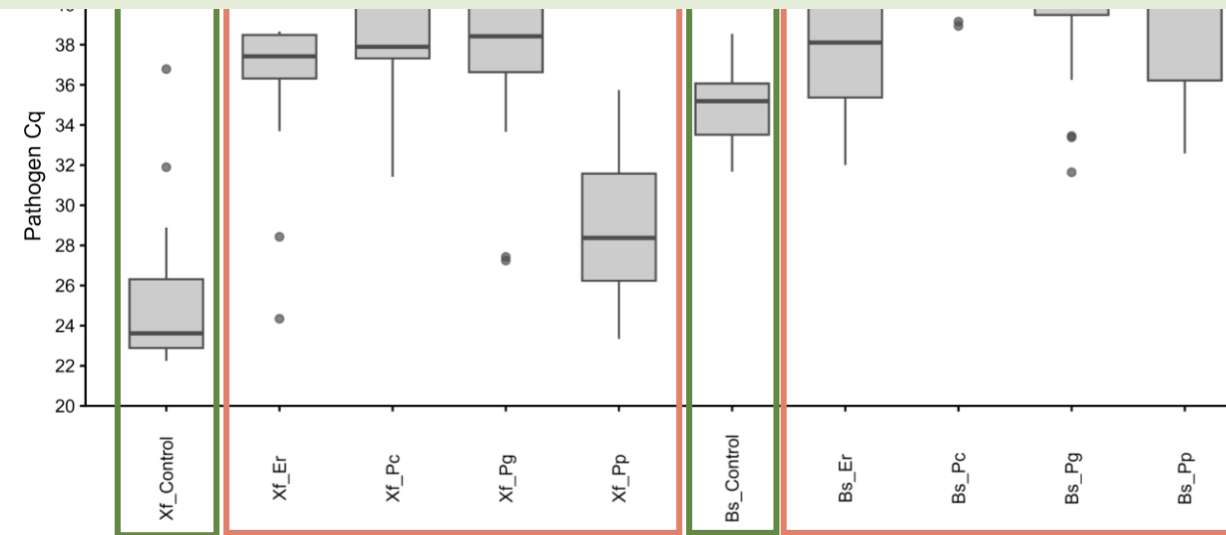
One-month assay



- **Lower** concentration of *X. fastidiosa* when co-inoculated with all endophytes except *P. phytostirmans*.
- **Lower** concentration of *B. salicis* when co-inoculated with all endophytes.

Reduction of pathogenic bacterial load in co-inoculation in most cases

- **Lower** concentration of *X. fastidiosa* when co-inoculated with all endophytes.
- **Lower** concentration of *B. salicis* when co-inoculated with 2 endophytes : *B. velezensis*, *P. coleopterorum*.

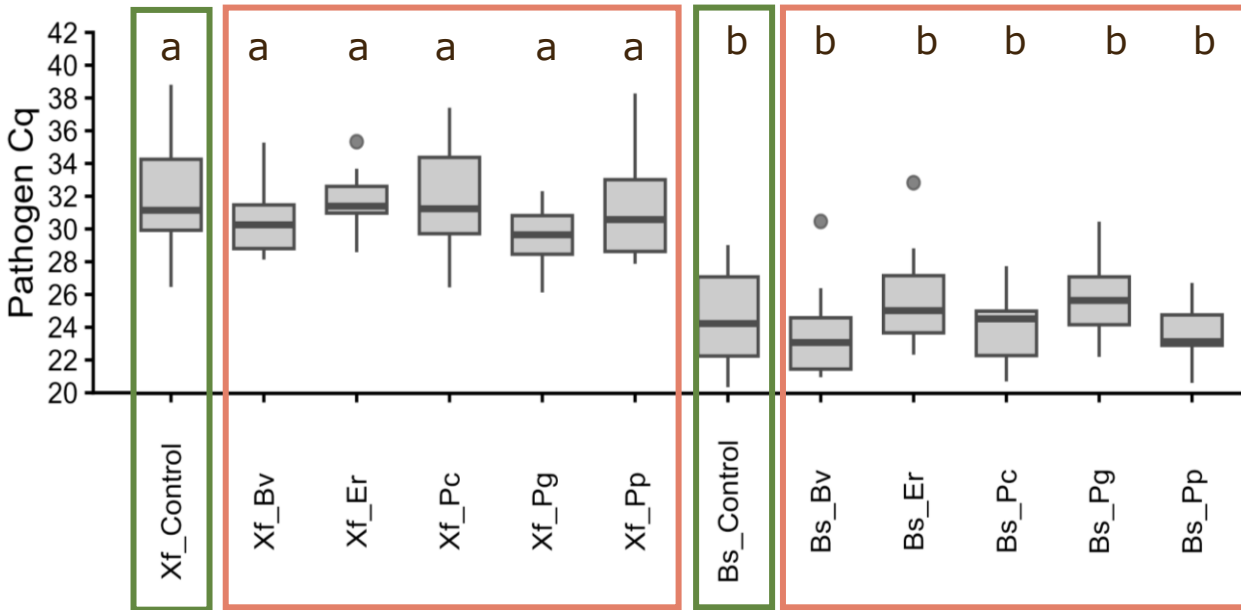


Three-month assay

PCR and qPCR detection

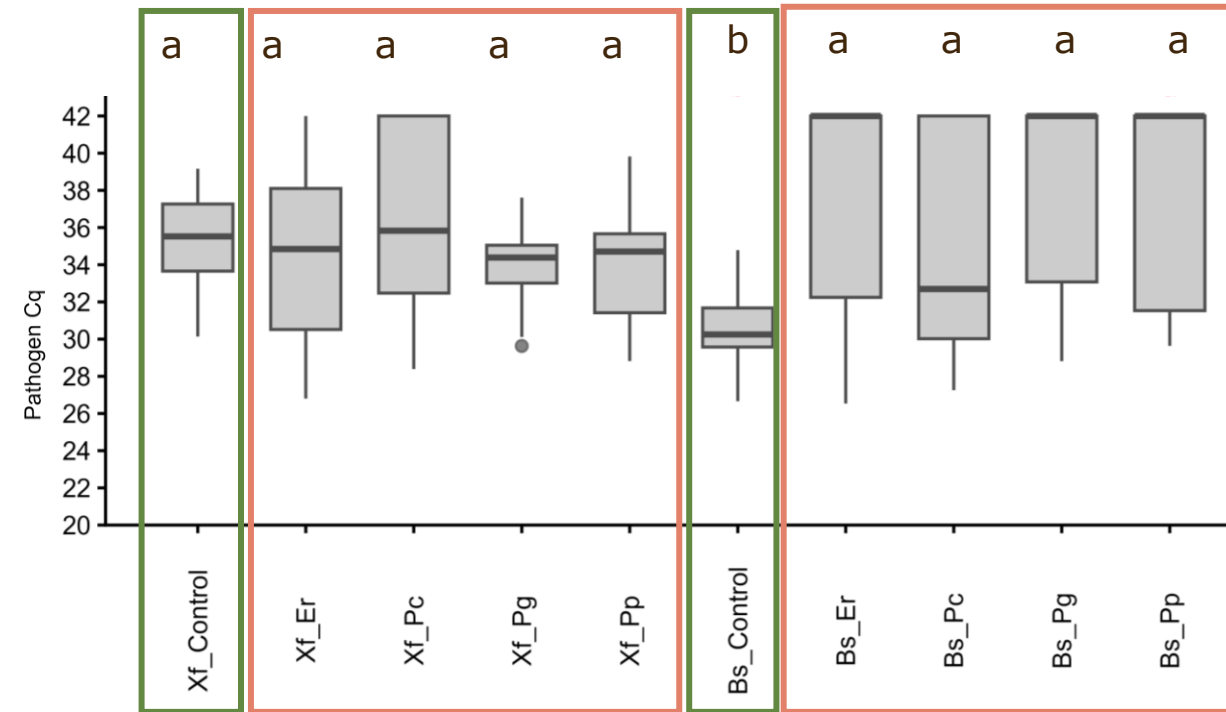
Willow assay

One-month assay



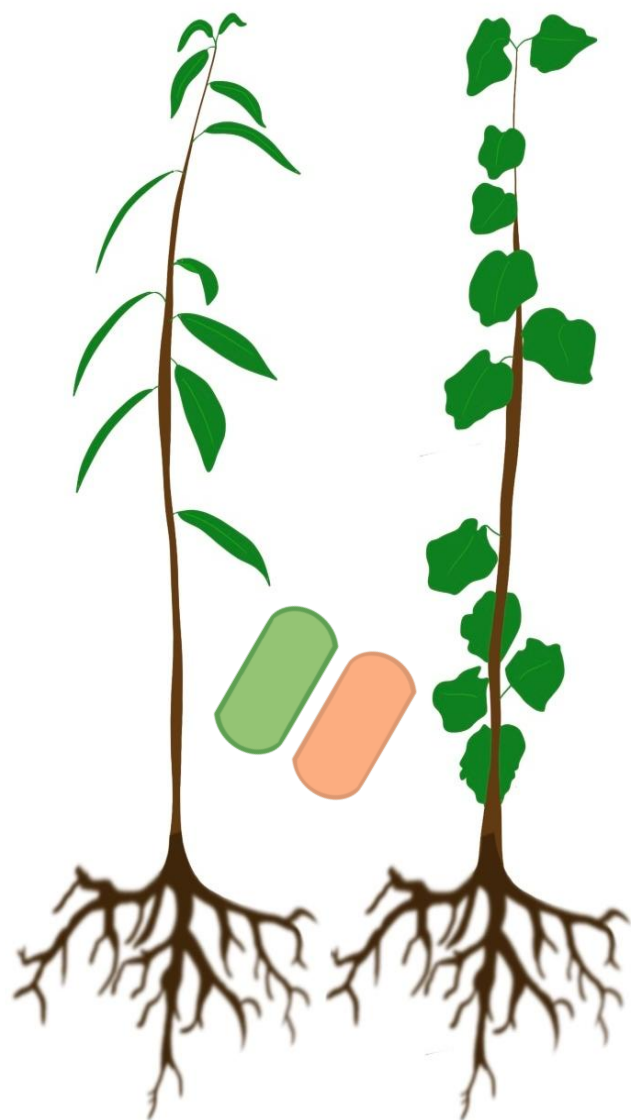
- No significant difference in pathogen load.

- No significant difference in *X. fastidiosa* load with any endophyte.
- Lower** concentration of *B. salicis* when co-inoculated with all endophytes.



Three-month assay

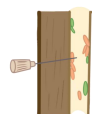
Xylem bacterial endophyte interacts with xylem-limited pathogen



- New description of *Salicaceae* xylem endophytome with
- 440 bacteria isolated from 43 genera and 104 species
 - 158 genera and 223 species sequenced



- 40 bacteria selected through *in vitro* confrontation
- 15 bacteria chosen from *in vitro* test for genome sequencing

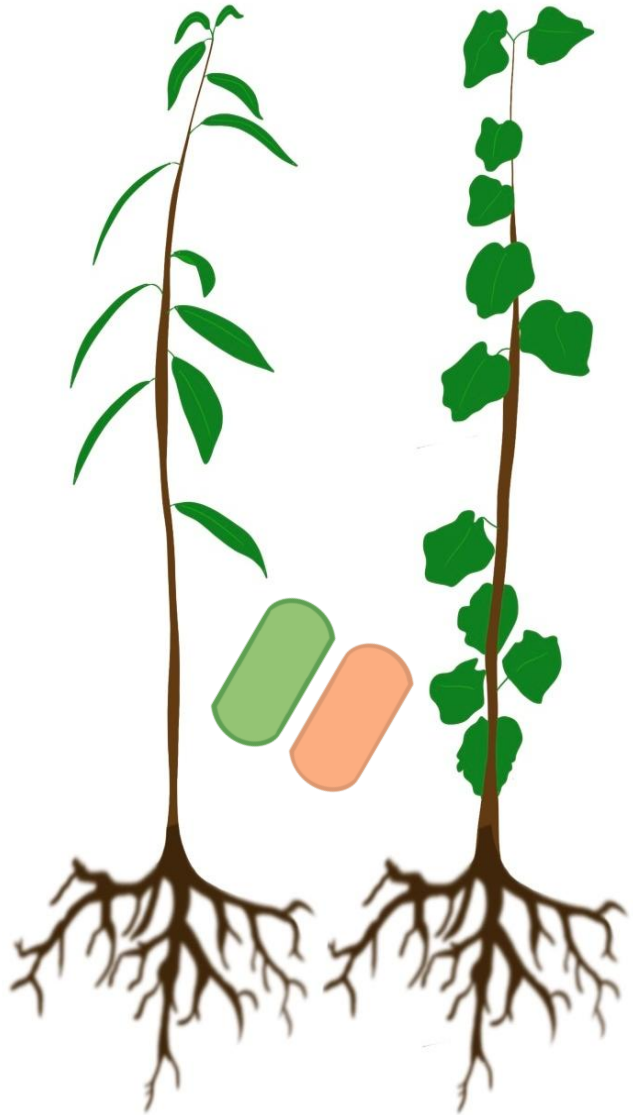


- 5 transformed bacteria used in co-inoculation
- New tools with fluorescent strains to track *in planta*

→ Effect of **co-inoculation** on **symptoms development** and **pathogen load** especially in tobacco after 1 month



What's next ?



Use metagenomics to better define *Salicaceae* endophytome



Use other *Salicaceae* species for co-inoculation assay



Use synthetic communities and not single inoculant



Use insect vector to disseminate synthetic communities

Supervisor: Claude Bragard (UCLouvain)

Transmission test : Noemi Casarin (UCLouvain)

Séverine Hasbroucq (ULB)

Mechanical inoculation : Lena Pesenti (UCLouvain)

Noemi Casarin (UCLouvain)

Louis Michot (UCLouvain)

Cyril Focant (Corder)

Amandine Gérardin (UCLouvain)

Isolation /identification : Lena Pesenti (UCLouvain)

Félicien Timmermans (UCLouvain)

Metabarcoding : Lena Pesenti (UCLouvain)

Manon Martin (UCLouvain)

In vitro test : Lena Pesenti (UCLouvain)

Héloïse DeBroux (HelHa)

François Marchandise (Corder)

Transformation : Lena Pesenti (UCLouvain)

Louis Michot (UCLouvain)

Thanks to all who provided material and support :

Dr. S. Lindow for KLN59.3 strain

Dr. Lionel Moulin for PsJN strain

BCCM LMG collection

CRA-w

Prof. Jean-Claude Grégoire

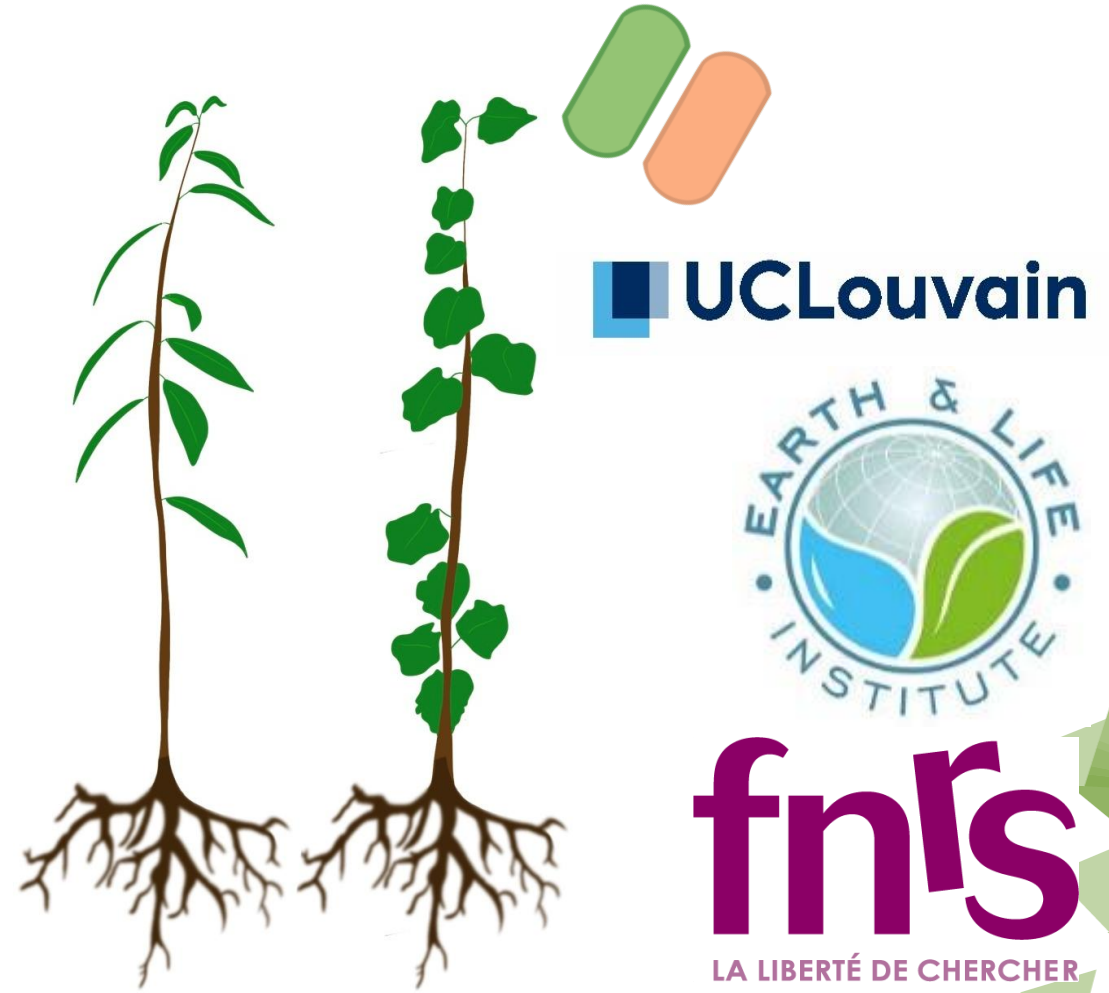
Thanks to FRIA – FNRS, UCLouvain and EFSA for funding.



Pesenti, 2026,
New Phytologist

Thank you for your attention !

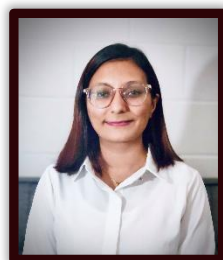
Any question welcome



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

Leonardo De La Fuente

Professor, Auburn University
Auburn, Alabama, USA



Mamata KC¹, Navdeep Kaur¹



A. Belviy¹



¹Department of Entomology and Plant Pathology, Auburn University.

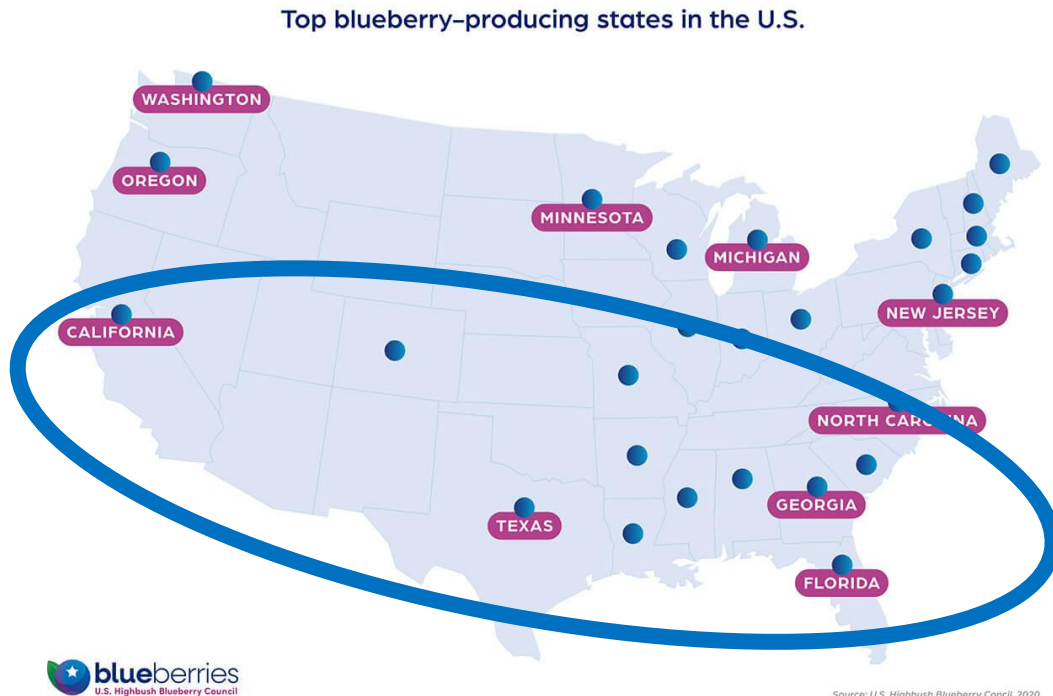
Blueberry: Bacteria Leaf Scorch

First reported in Georgia (USA) in 2009

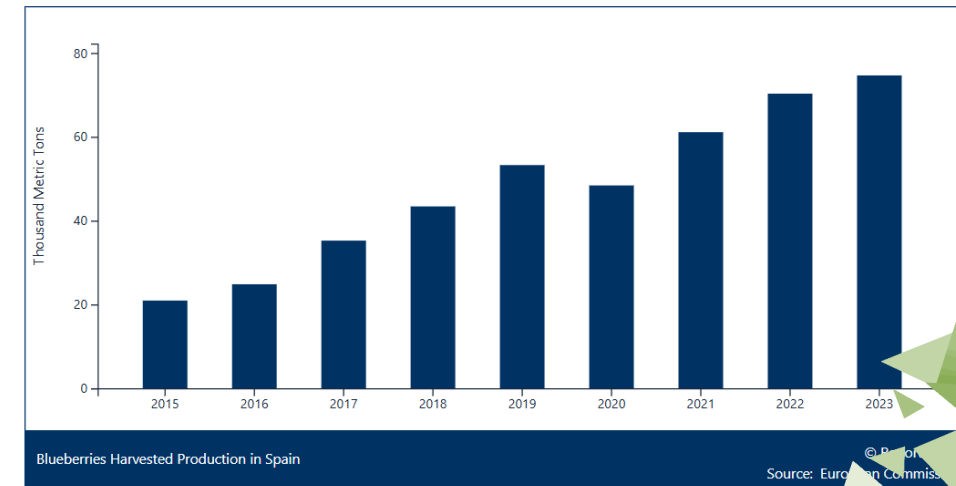


Low chill Blueberry varieties: Southern highbush and rabbiteye

Southern States in the US



Huelva (#1) in Spain



Two Hosts: Grapevine and Blueberry



Blueberry pathosystem

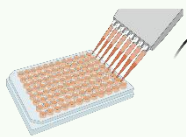
In planta assays

Disease severity and bacterial colonization



In vitro assays

Growth and biofilm formation in blueberry sap



Grapevine pathosystem

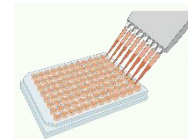
In planta assays

Disease severity and bacterial colonization



In vitro assay

Growth, biofilm formation, and adaptation to grapevine sap



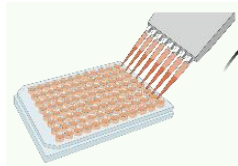


Blueberry pathosystem



***In planta* assays**

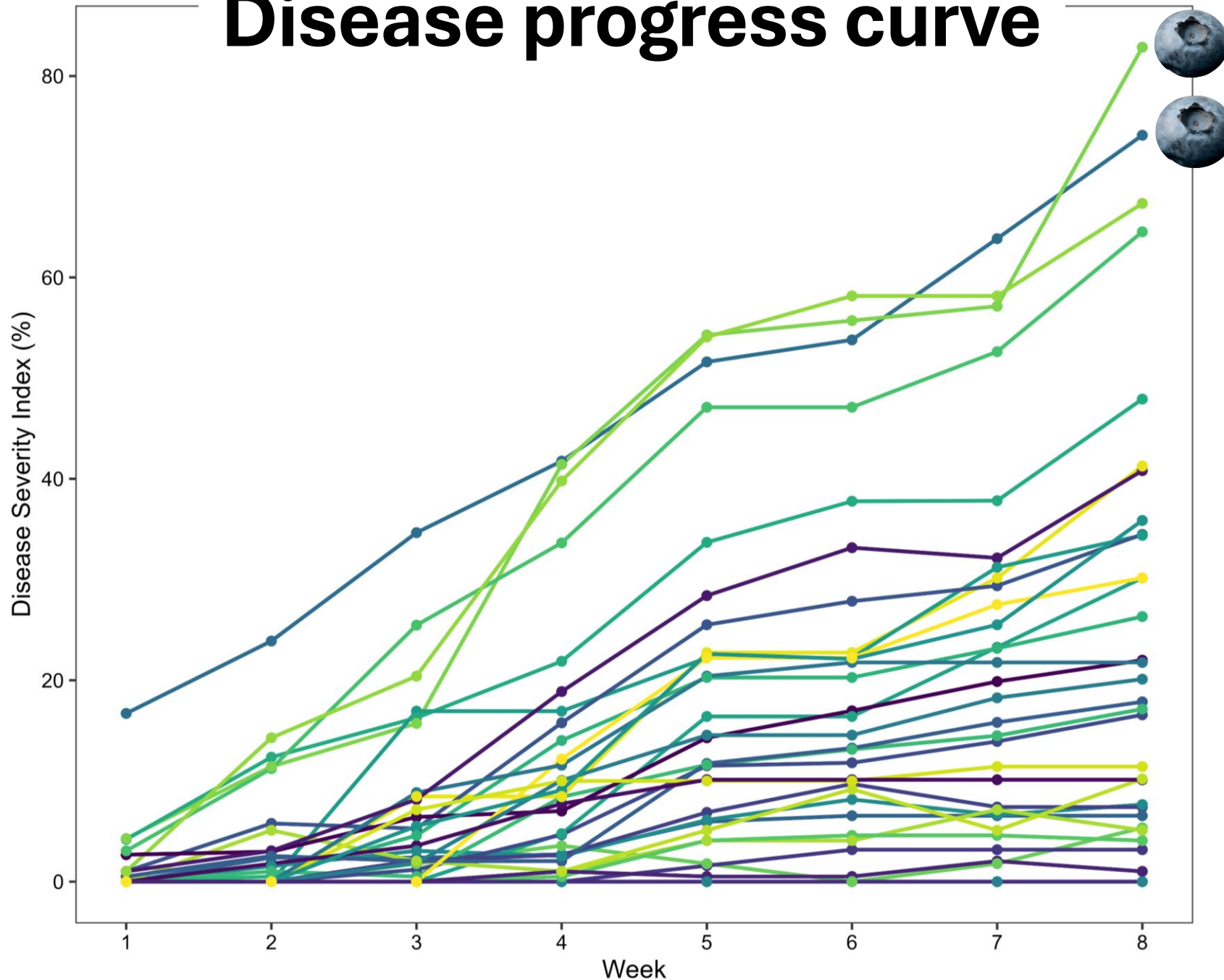
Disease severity and bacterial colonization



***In vitro* assays**

Growth and biofilm formation in blueberry sap

Disease progress curve



31 strains tested

17 EU

3 Intercepted

7 US Blueberry

4 US others

Strain

AlmaEm3	IVIA5387.2
AlmaReb1	IVIA5770
AlmaReb3	IVIA5901
B3SN17-4	IVIA6629
BB08-1	LAY3C
BBI64	PBS
CCPM1	PierceMed1
CFBP8073	TemeculaL
CFBP8173	TOS4
CFBP8356	WM1-1
CIST-18	XF3348
CO33	XYL1961
DeDonno	XYL1966
ESVL	XYL2014
IVIA11529	XYL466
IVIA5235	XYL468

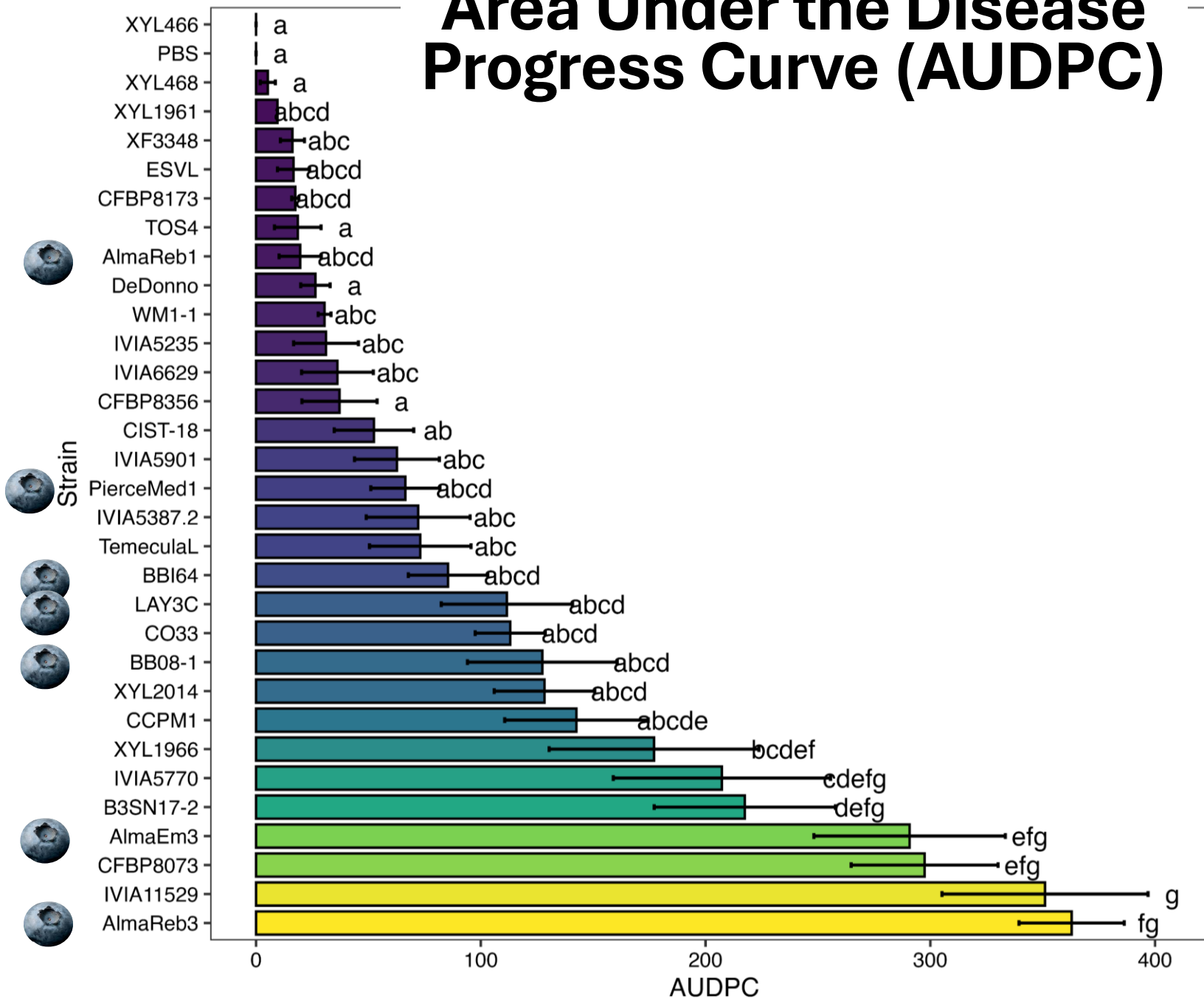
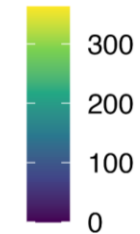
Area Under the Disease Progress Curve (AUDPC)

31 strains tested

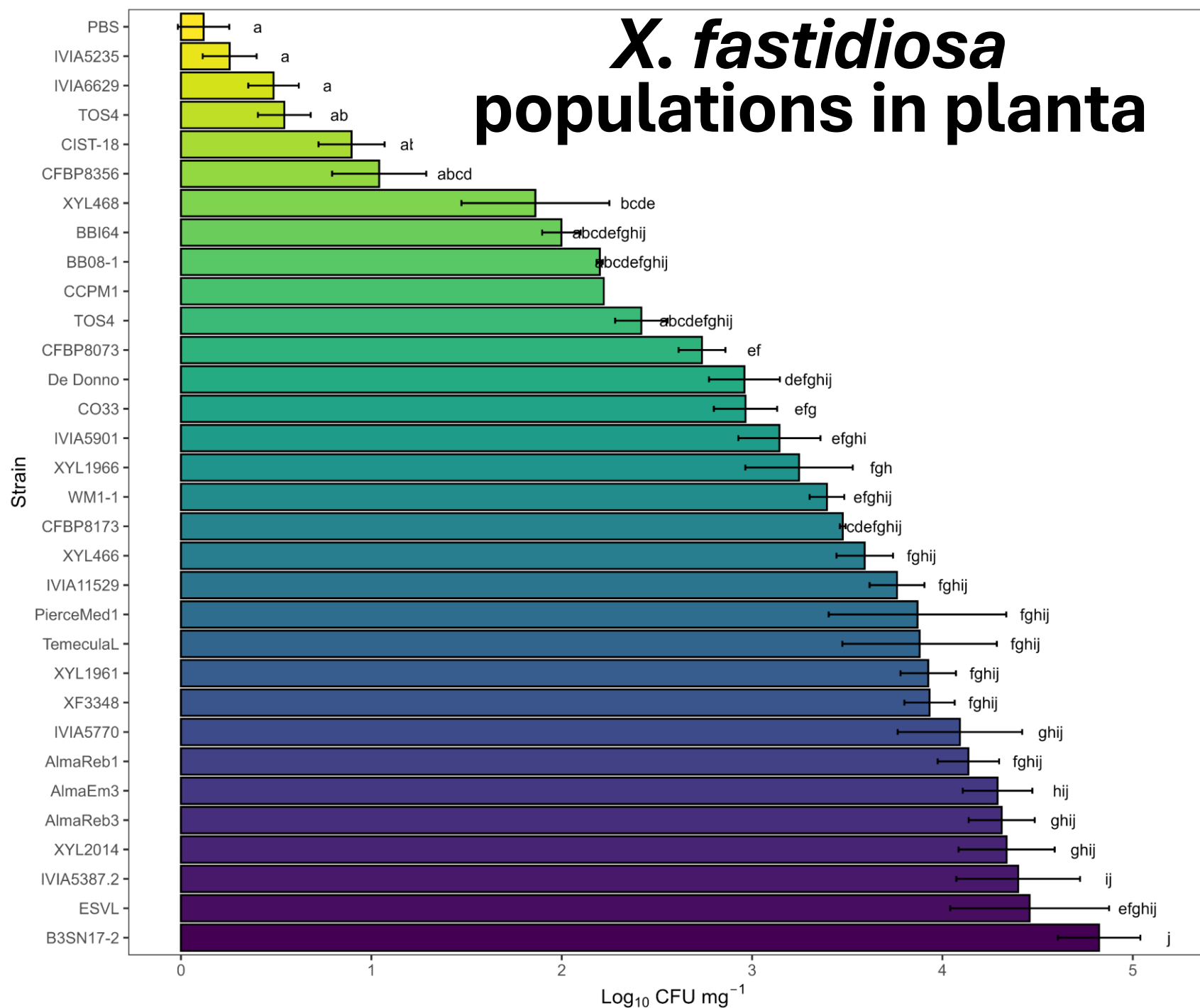
17 EU

3 Intercepted
7 US Blueberry
4 US others

AUDPC



X. fastidiosa populations in planta



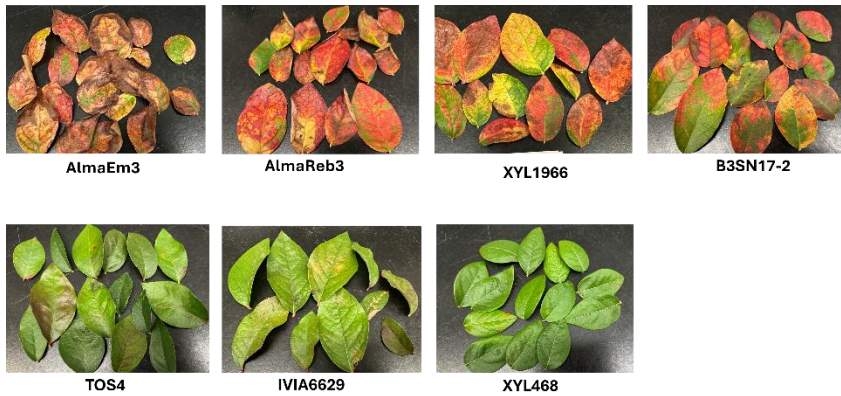
31 strains tested

17 EU

3 Intercepted
7 US Blueberry
4 US others

All subspecies can cause symptoms in blueberries

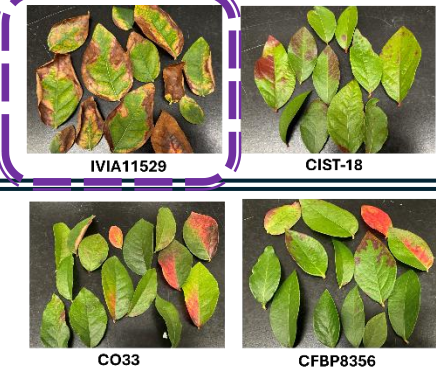
Xf subsp. *multiplex*



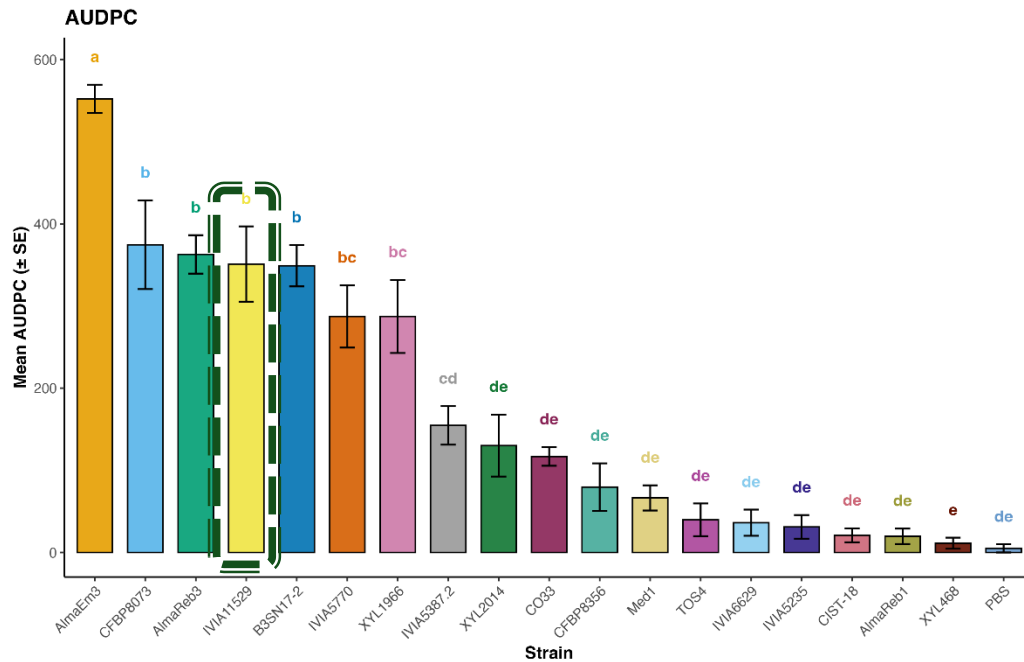
Xf subsp. *fastidiosa*



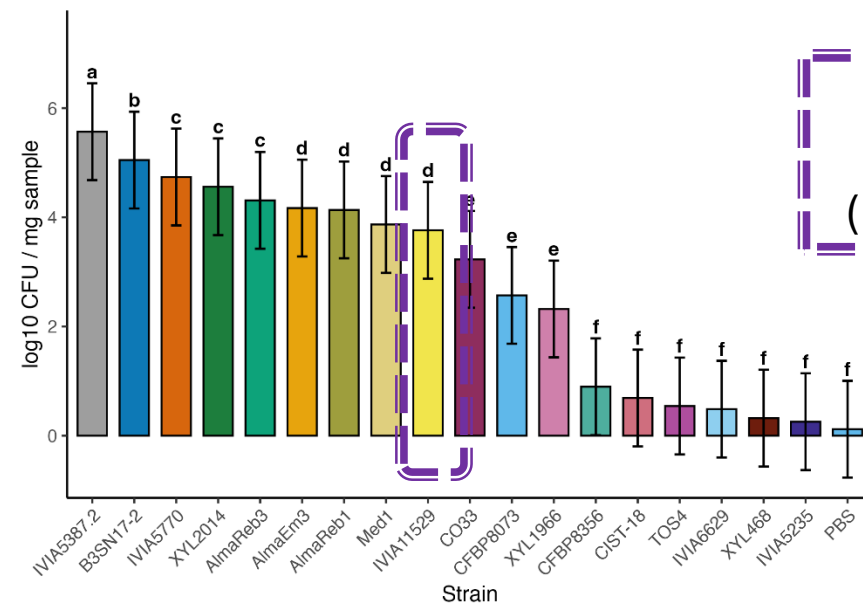
Xf subsp. *pauca*



Xf subsp. *sandyi*



qPCR bacterial load by strain



IVIA11529:
Lavander
(Ibiza, Spain)

Growth in blueberry sap

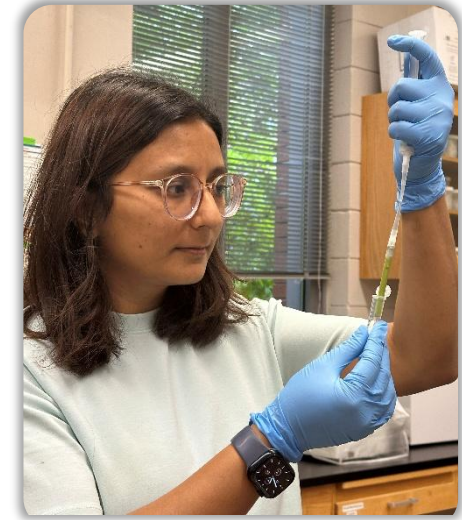


- Difficult to collect: 1-1.5 ml of sap/plant
- Used ~130 plants grown in the greenhouse for sap



Negative pressure
xylem extraction unit

(Modified from Longchar et.al, 2020)



Growth Media

PD3

PD3 +
Susceptible Blueberry Sap
(25%)

PD3 + Resistant Blueberry Sap
(25%)

X. fastidiosa strains on blueberry sap (susceptible cv 'Rebel')



Xf subsp. *multiplex*

AlmaEm3, AlmaReb3, TOS4,
XYL466, XYL468

Xf subsp. *fastidiosa*

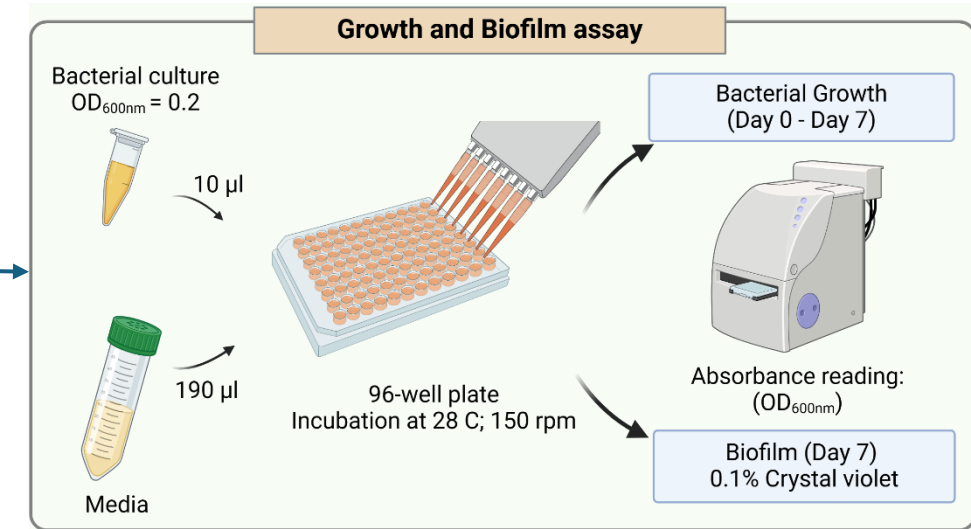
AlmaReb1, WM1-1, IVIA5387.2,
IVIA5770, CFBP8073, Med1,
TemeculaL

Xf subsp. *sandyi*

CFBP8356

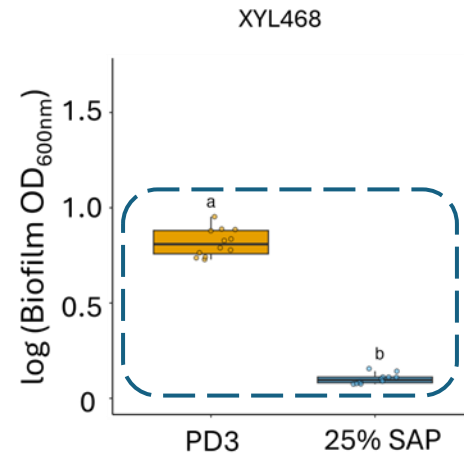
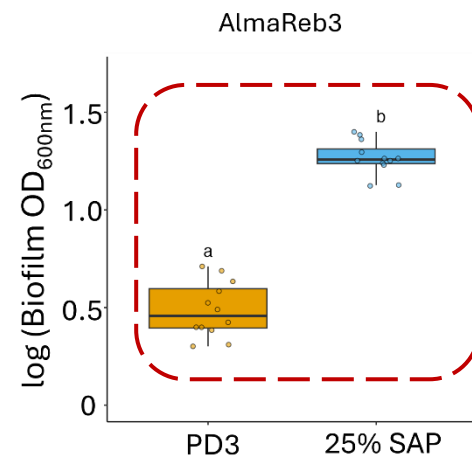
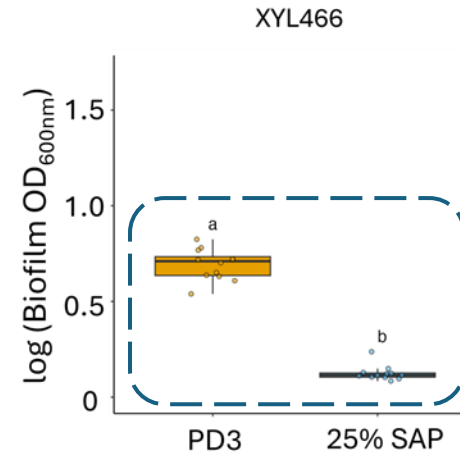
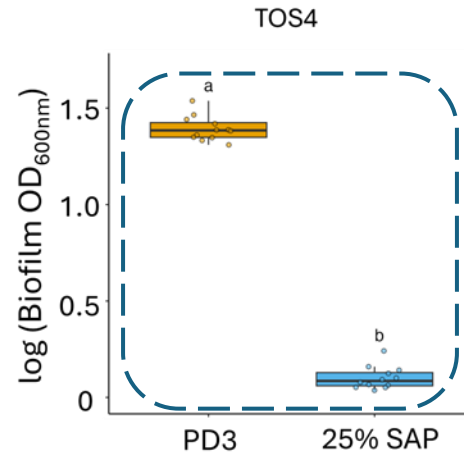
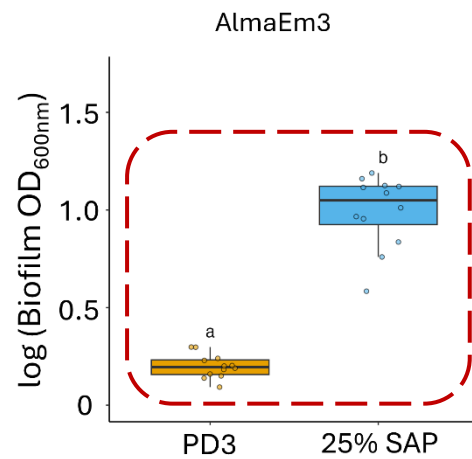
→ Blueberry Sap →

In vitro assay: 25% Blueberry sap vs. PD3

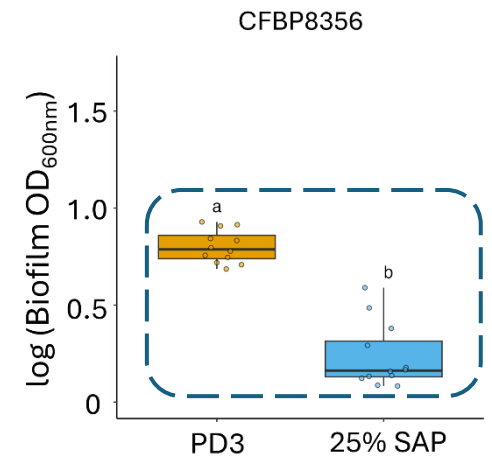


X. fastidiosa strains on blueberry sap (susceptible cv 'Rebel')

Xf subsp. *multiplex*

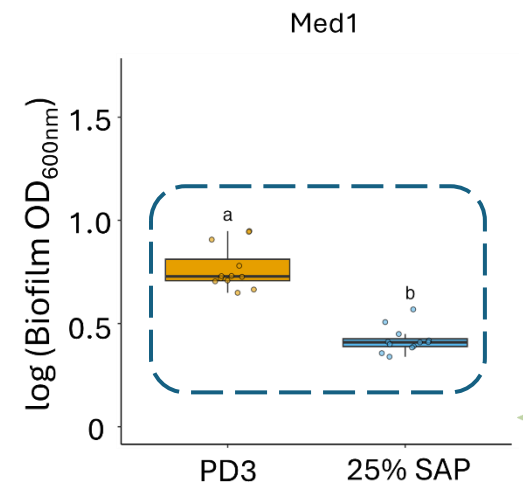
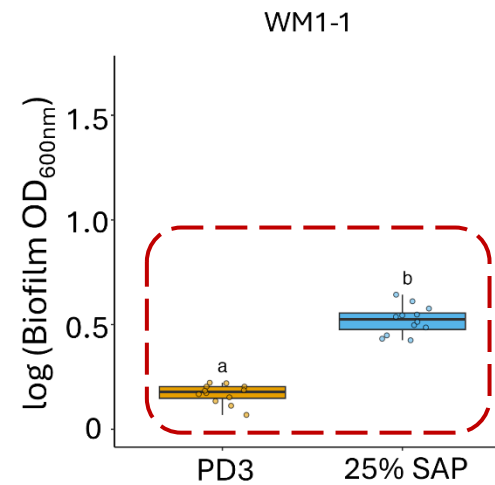
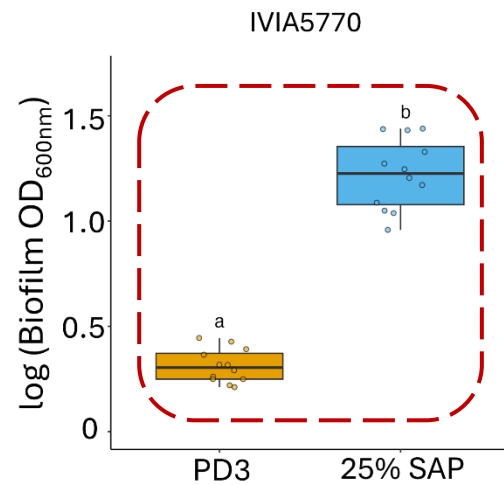
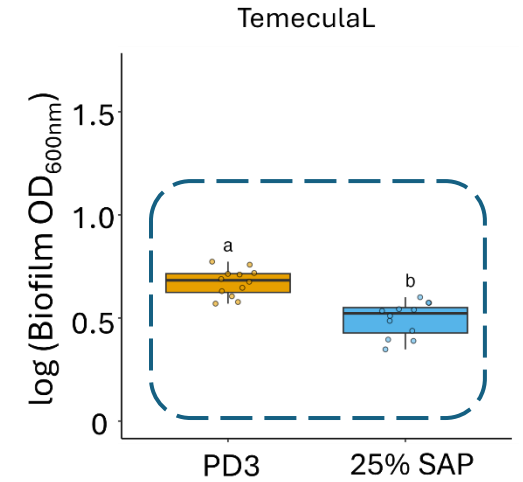
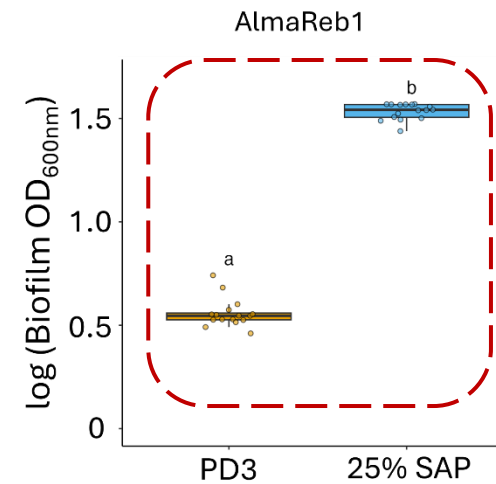
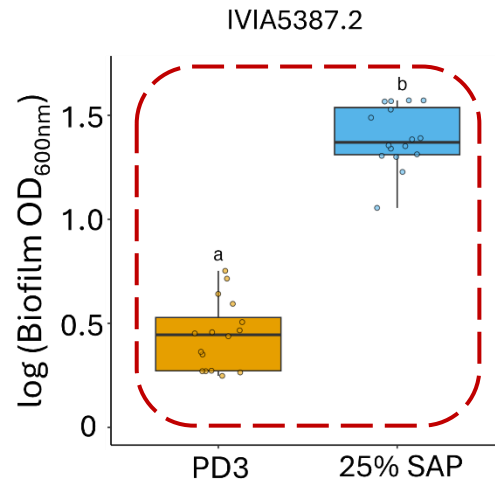
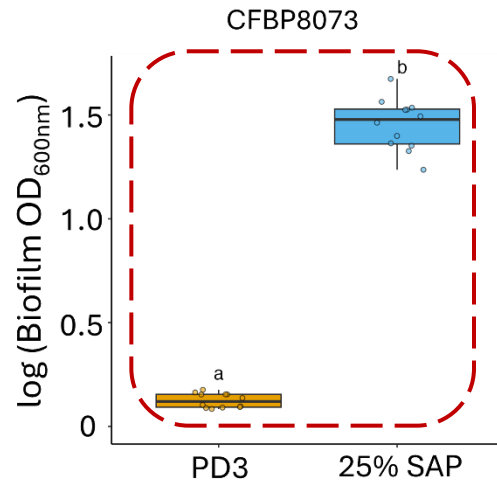


Xf subsp. *sandyi*



X. fastidiosa strains on blueberry sap (susceptible cv 'Rebel')

Xf subsp. *fastidiosa*



Biofilm formation in blueberry sap is indicative of symptoms in planta



AlmaEm3



AlmaReb3



CFBP8073



IVIA5387.2



IVIA5770



WM1-1 **

Biofilm ↑ → Severe symptoms



TOS4



XYL468



CFBP8356



XYL466 **



Med1

Biofilm ↓ → Mostly asymptomatic



AlmaReb1

Biofilm ↑
Mostly asymptomatic



TemeculaL **

Biofilm ↓
Mild symptoms

Correlation symptoms/biofilm in sap: ~85%

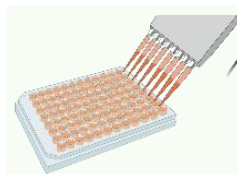


Grapevine pathosystem



***In planta* assays**

Disease severity and bacterial colonization



***In vitro* assay**

Growth, biofilm formation, and adaptation to grapevine sap

Virulence in grapevine

Grapevine-isolated strains

Xf subsp. *fastidiosa*
TemeculaL, WM1, CCPM1, IVIA5770

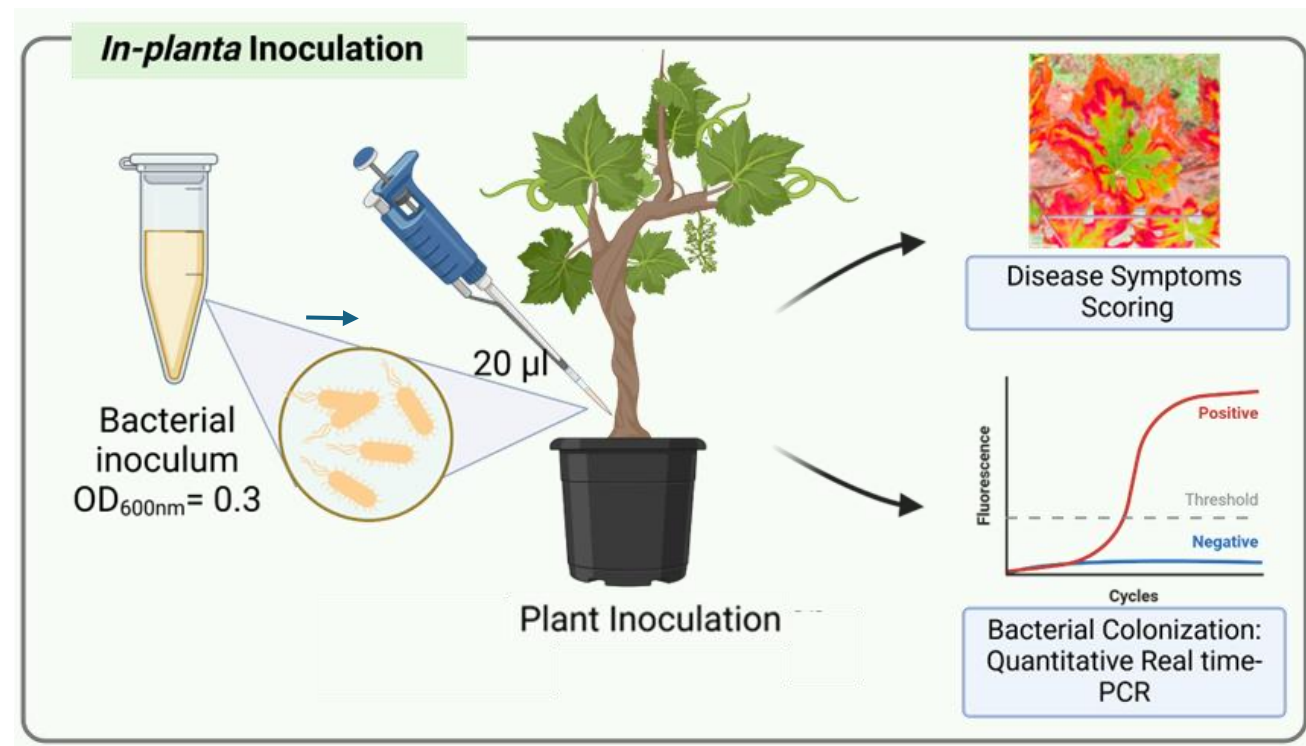
Blueberry- isolated strains

Xf subsp. *multiplex*
AlmaEm3, BB08

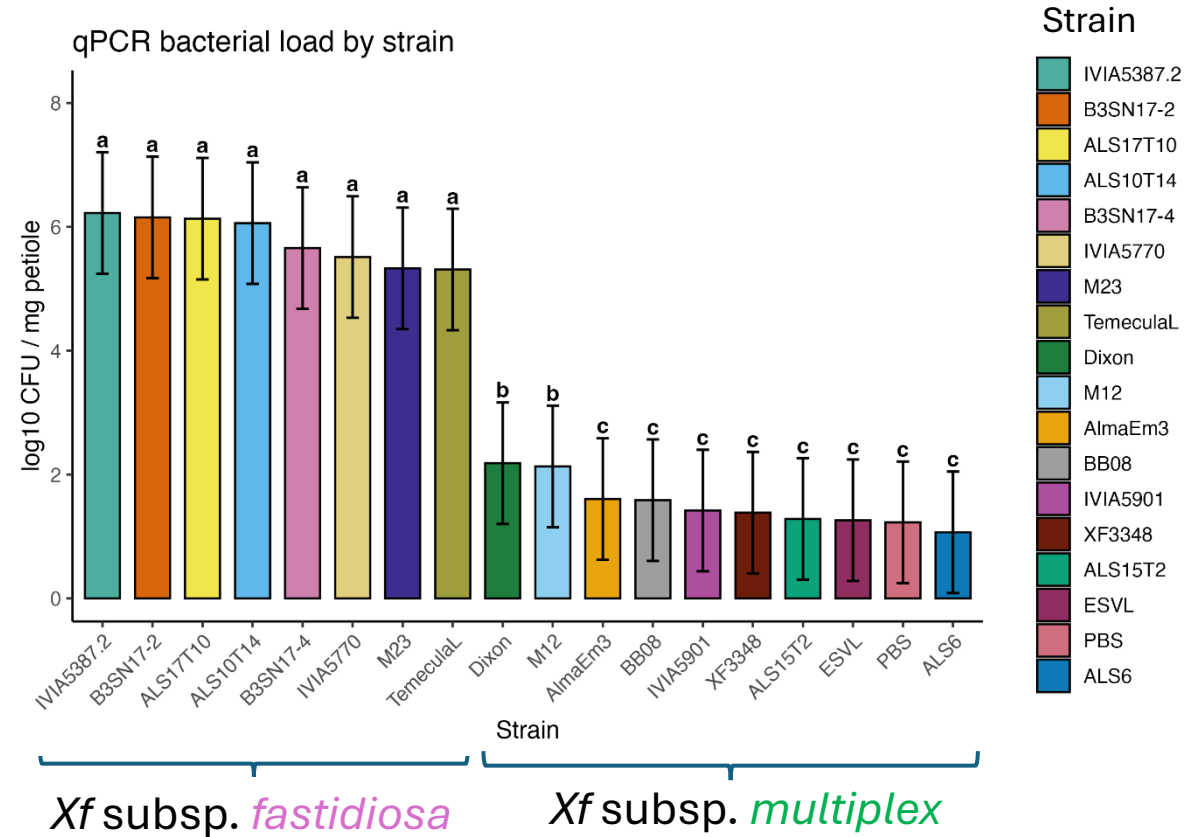
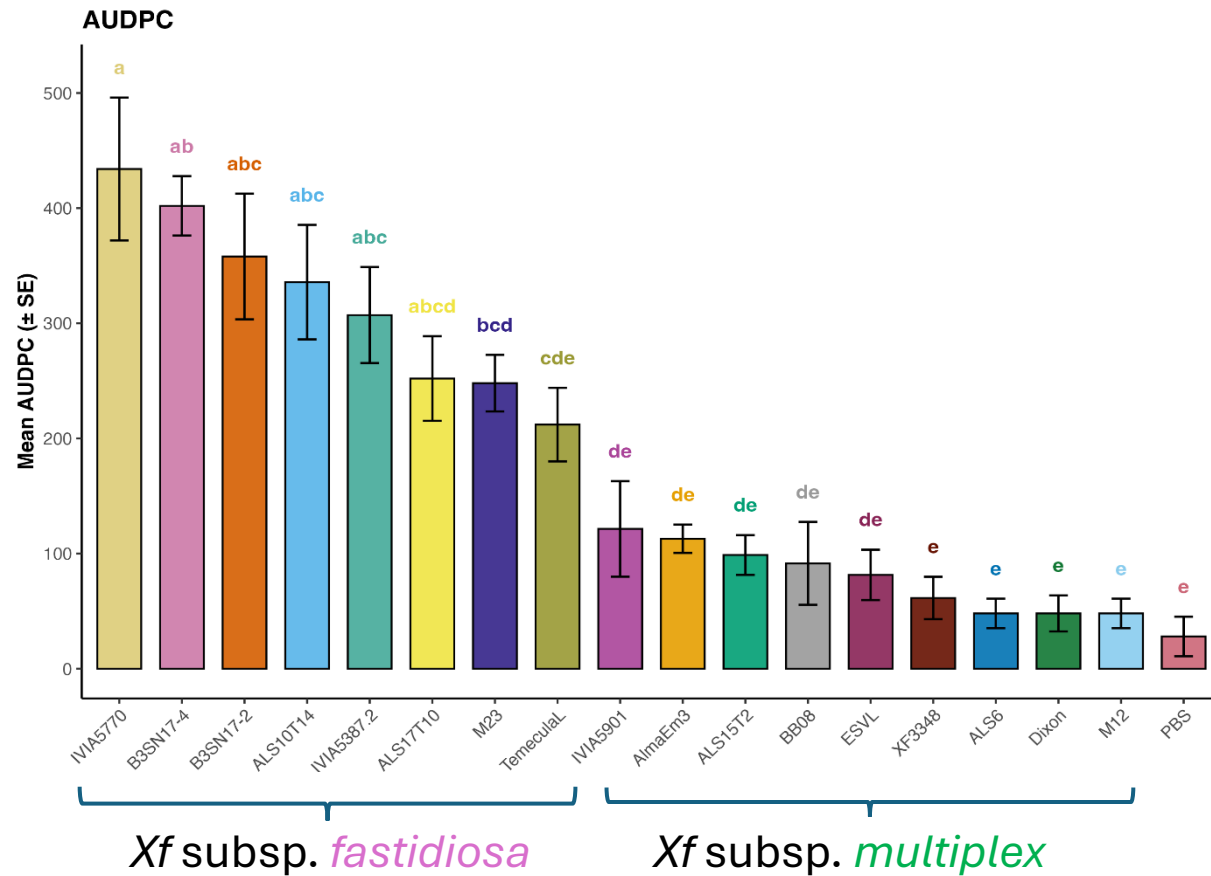
Almond- isolated strains

Xf subsp. *multiplex*
ALS15T2, IVIA5901, XF3348,
ESVL, Dixon, ALS6, M12

Xf subsp. *fastidiosa*
B3SN17-2, B3SN17-4, M23,
ALS10T14, ALS17T10, IVIA5387.2



Only almond and grape *Xf* subsp. *fastidiosa* strains caused symptoms



AUDPC correlates with bacterial colonization *in-planta*

Grapevine sap collection in the field



Grape sap dripping from
freshly cut canes

Growth Media

PD3

PD3 +
Grapevine Sap
(Susceptible 'Chardonnay')
(25%, 50%, 75%)

PD3 +
Grapevine Sap
(Resistant 'Blanc Du Bois')
(25%)

X. fastidiosa strains on grapevine sap (susceptible cv 'Chardonnay')

Grapevine-isolated strains

Xf subsp. *fastidiosa*
TemeculaL, WM1, CCPM1, IVIA5770

Blueberry- isolated strains

Xf subsp. *multiplex*
AlmaEm3, BB08

Almond- isolated strains

Xf subsp. *multiplex*
ALS15T2, IVIA5901, XF3348,
ESVL, Dixon, ALS6, M12
Xf subsp. *fastidiosa*
B3SN17-2, B3SN17-4, M23,
ALS10T14, ALS17T10, IVIA5387.2

Grape Sap

In vitro assay
PD3 vs. 25%, 50%, 75% Sap

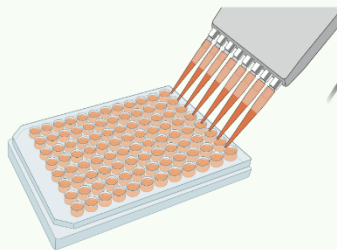
Growth and Biofilm assay

Bacterial culture
 $OD_{600nm} = 0.2$

10 μ l

190 μ l

Media



96-well plate
Incubation at 28 C; 150 rpm

Bacterial Growth
(Day 0 - Day 7)



Absorbance reading:
(OD_{600nm})

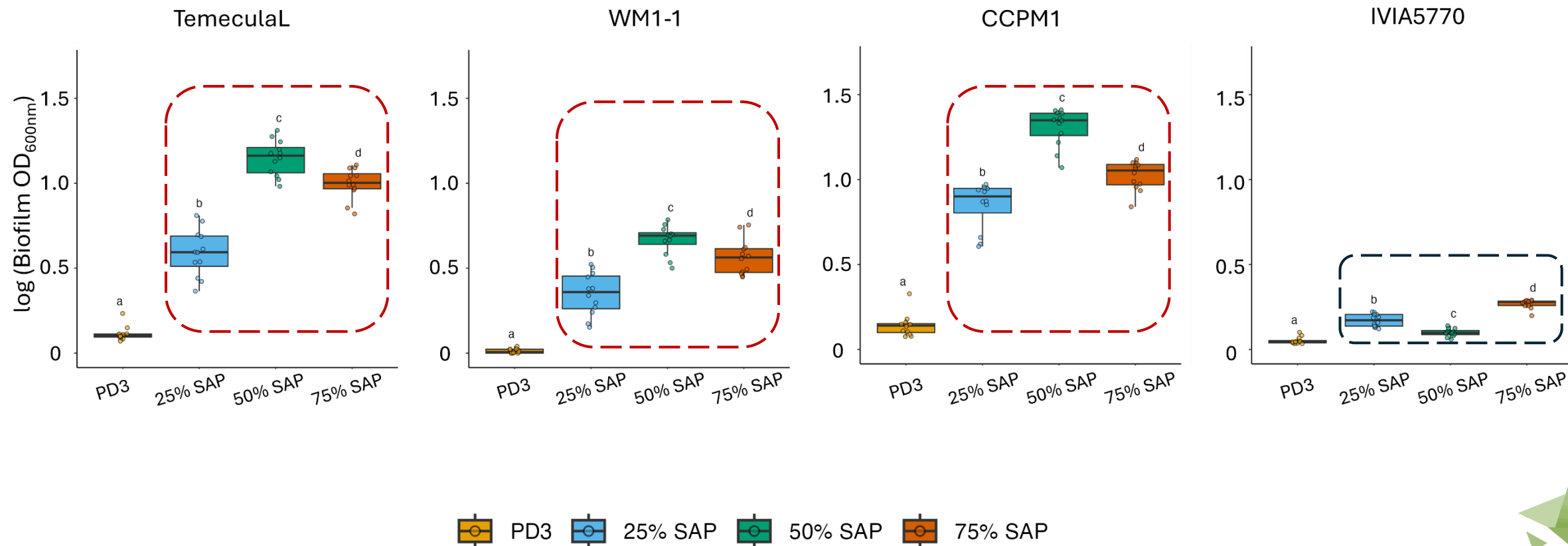
Biofilm (Day 7)
0.1% Crystal violet

X. fastidiosa strains on grapevine sap (susceptible cv 'Chardonnay')

Grapevine-isolated strains

In vitro assay

PD3 vs. 25%, 50%, 75% Sap

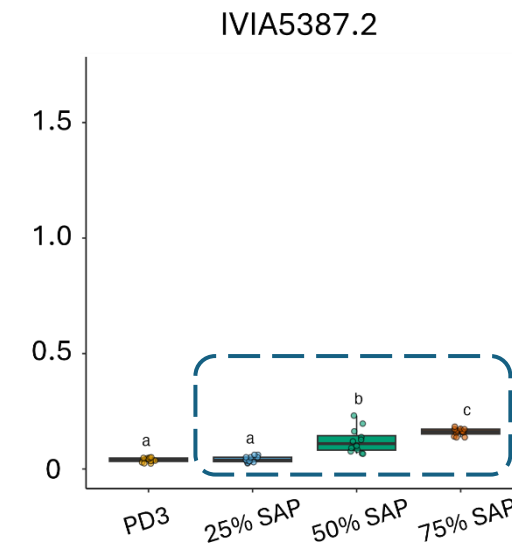
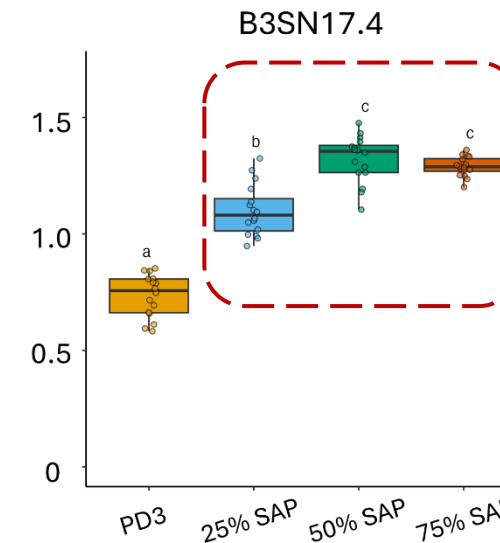
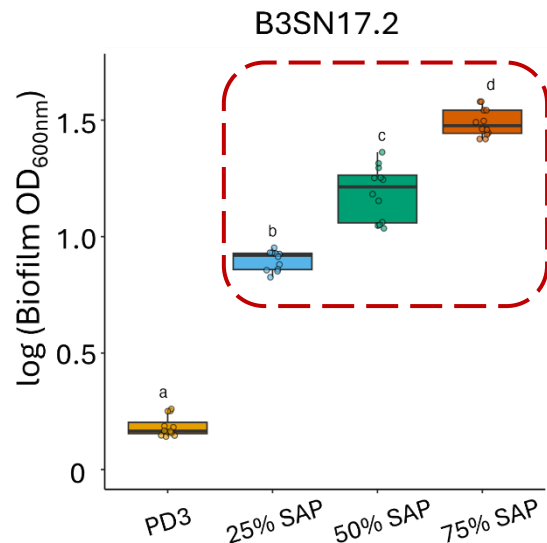
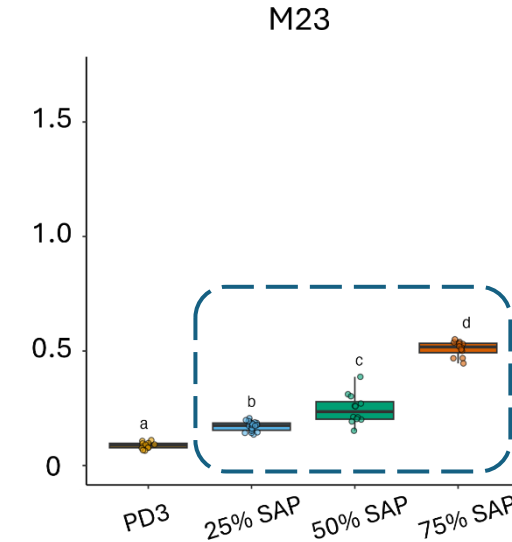
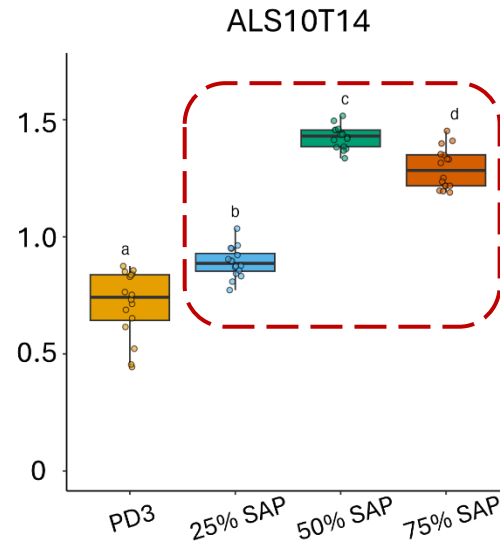
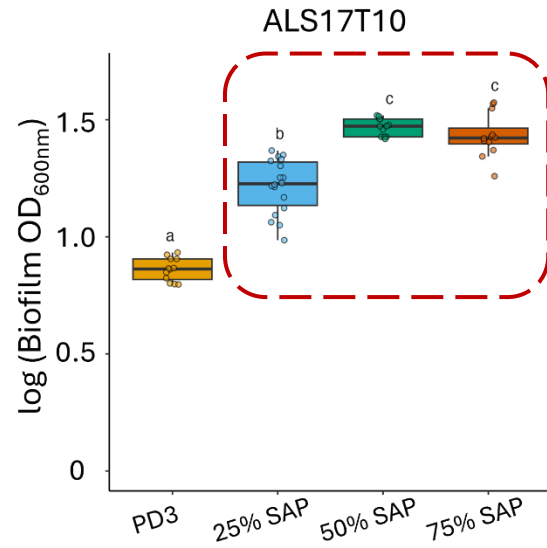


X. fastidiosa strains on grapevine sap (susceptible cv 'Chardonnay')

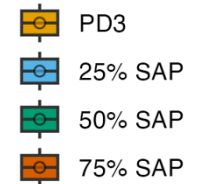


Almond-isolated strains

Xf subsp. *fastidiosa*



Media

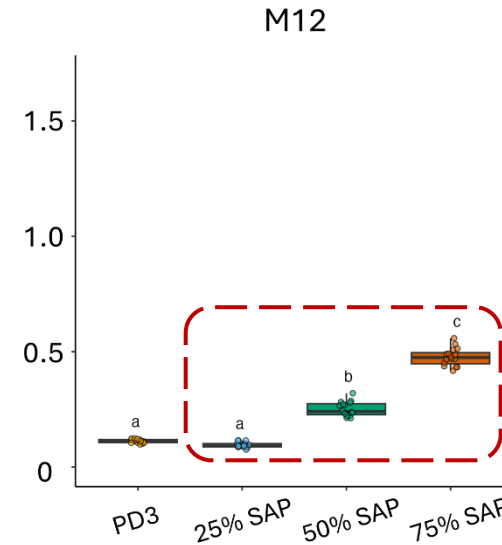
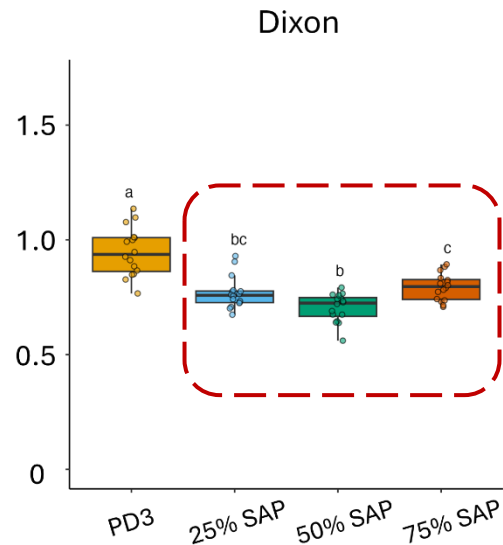
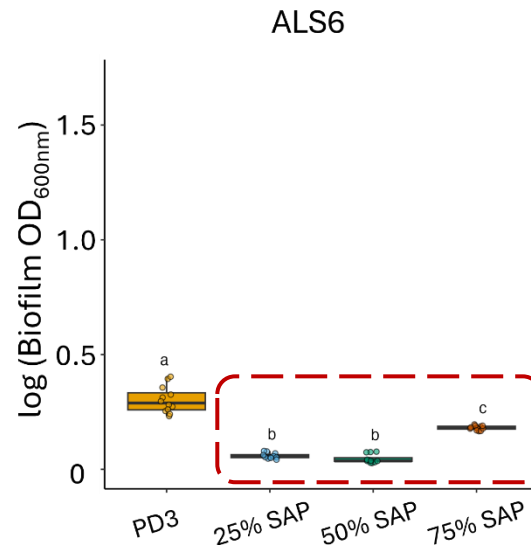
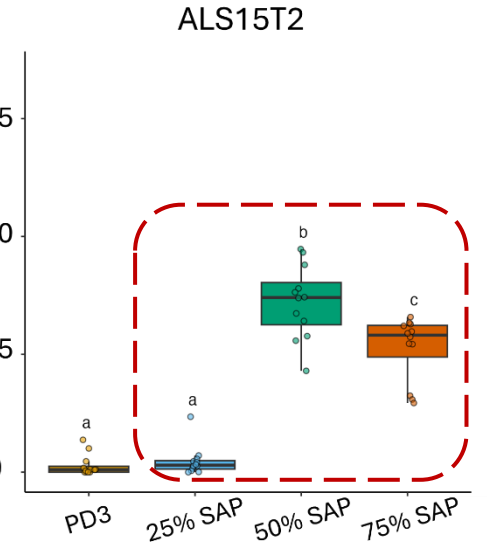
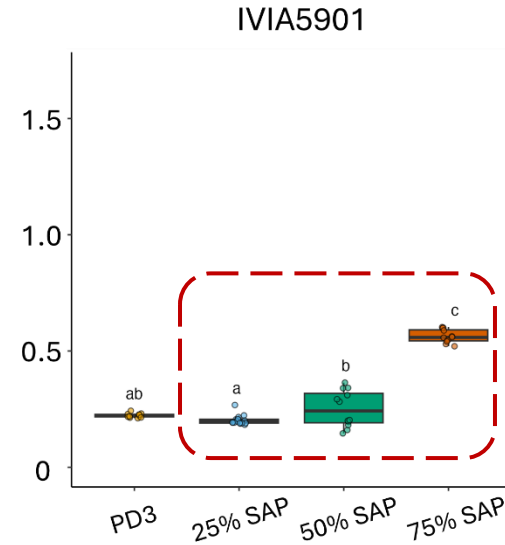
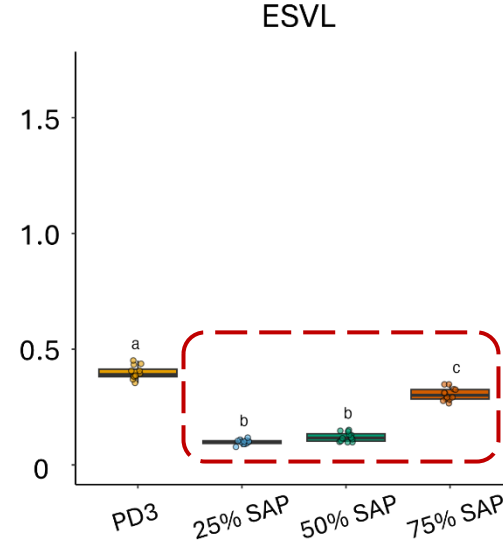
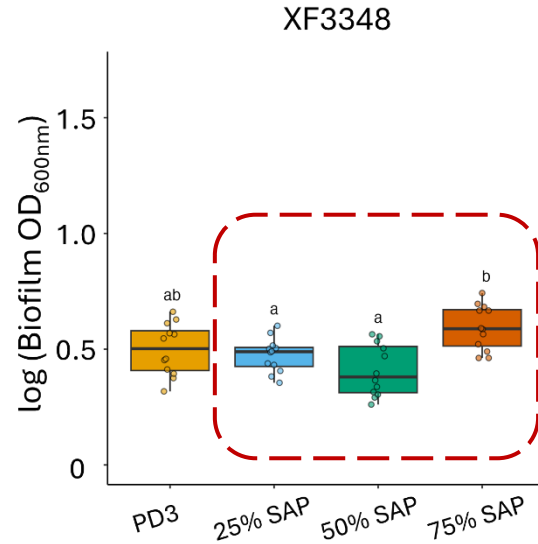


X. fastidiosa strains on grapevine sap (susceptible cv 'Chardonnay')

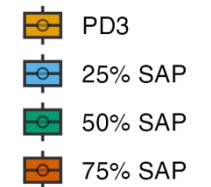


Almond- isolated strains

Xf subsp. *multiplex*



Media

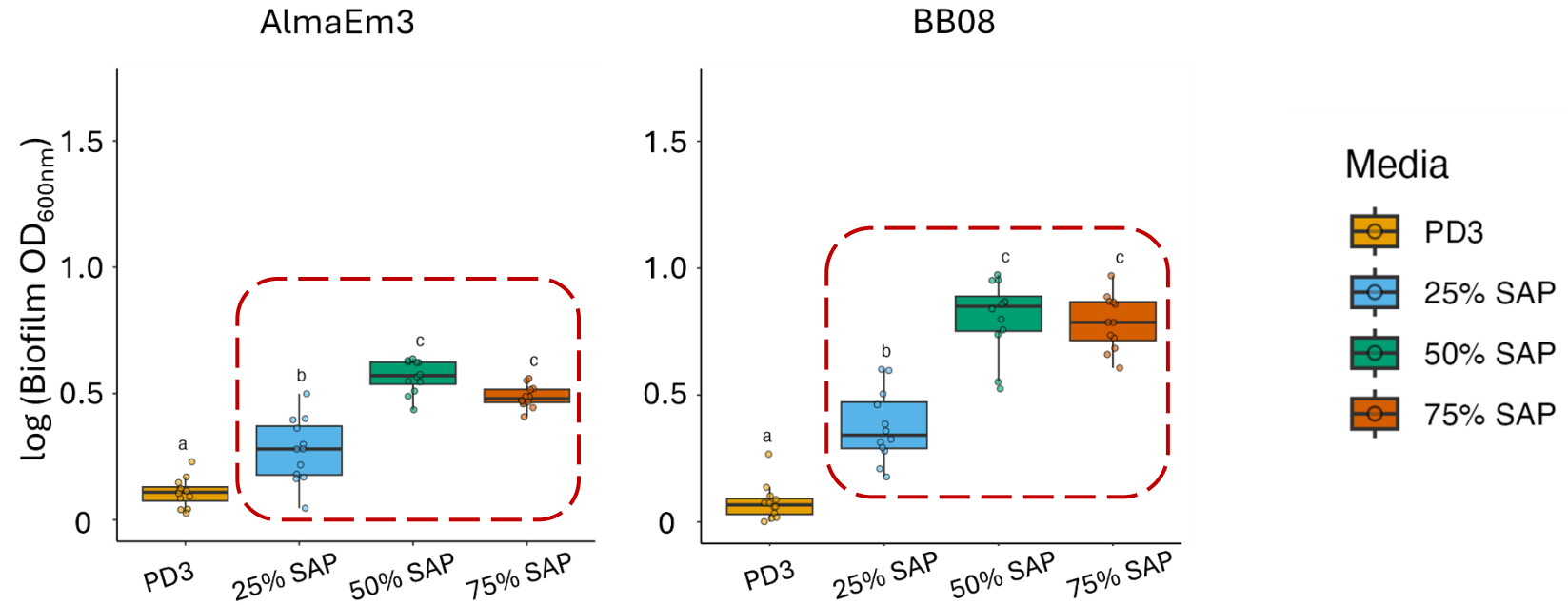


X. fastidiosa strains on grapevine sap (susceptible cv 'Chardonnay')



Xf subsp. *multiplex*

Blueberry- isolated strains



Only *X. fastidiosa* subsp. *fastidiosa* caused disease on grapevines

Xf subsp. *fastidiosa*



TemeculaL, WM1,
CCPM1



B3SN17-2, B3SN17-4,
ALS10T14, ALS17T10

High biofilm → Severe symptoms



IVIA5770



IVIA5387.2, M23

Low Biofilm → Severe symptoms

Xf subsp. *multiplex*



IVIA5901, XF3348, ESVL
Dixon, ALS6, M12

Low Biofilm → Asymptomatic



AlmaEm3, BB08



ALS15T2

High biofilm → Asymptomatic

Correlation symptoms/biofilm in sap: ~70%



Susceptible vs. resistant sap



Biofilm of *X. fastidiosa* strains on Susceptible vs Resistant blueberry sap



Blueberry

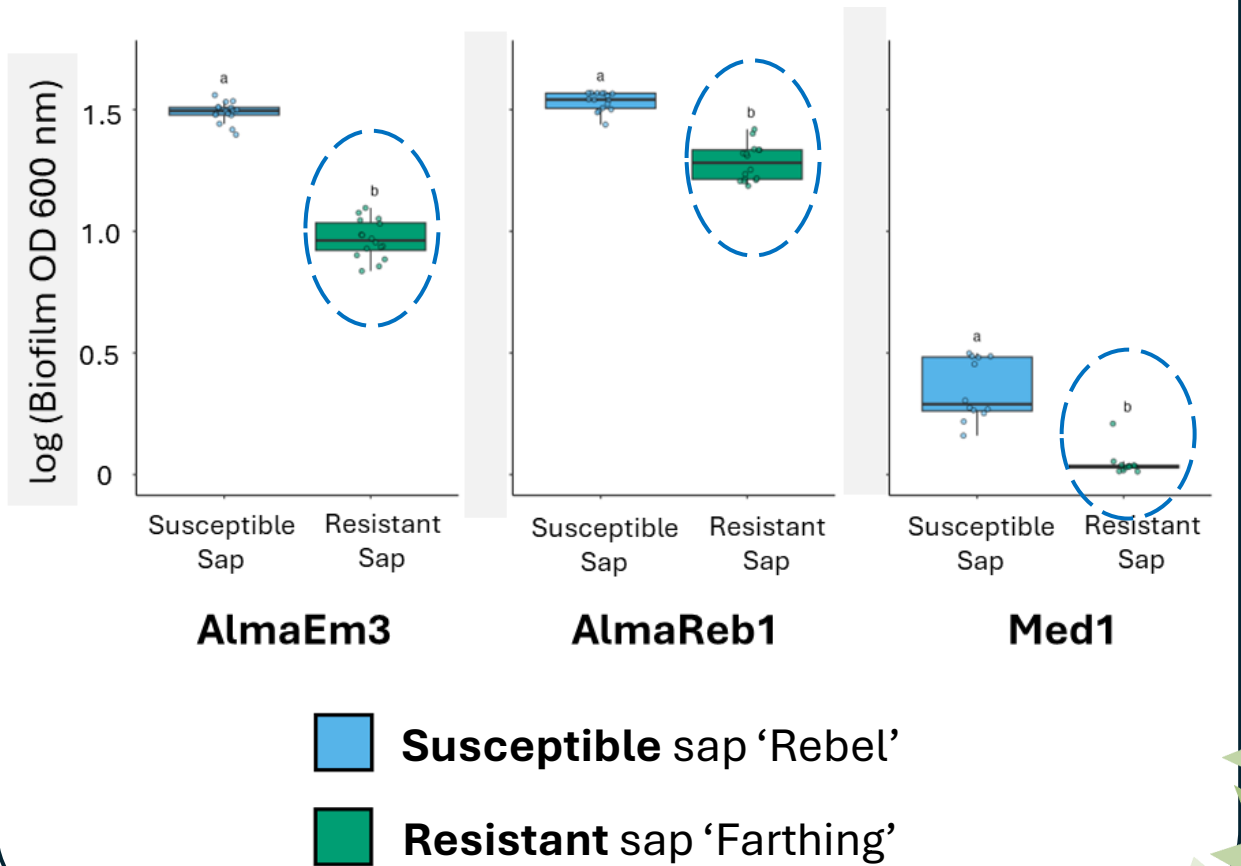
Susceptible Cultivar: Rebel

Resistant Cultivar: Farthing

Blueberry-Isolated Strains:
AlmaEm3, AlmaReb1, Med1



Blueberry



Biofilm of *X. fastidiosa* strains on Susceptible vs Resistant grapevine sap



Grapevine

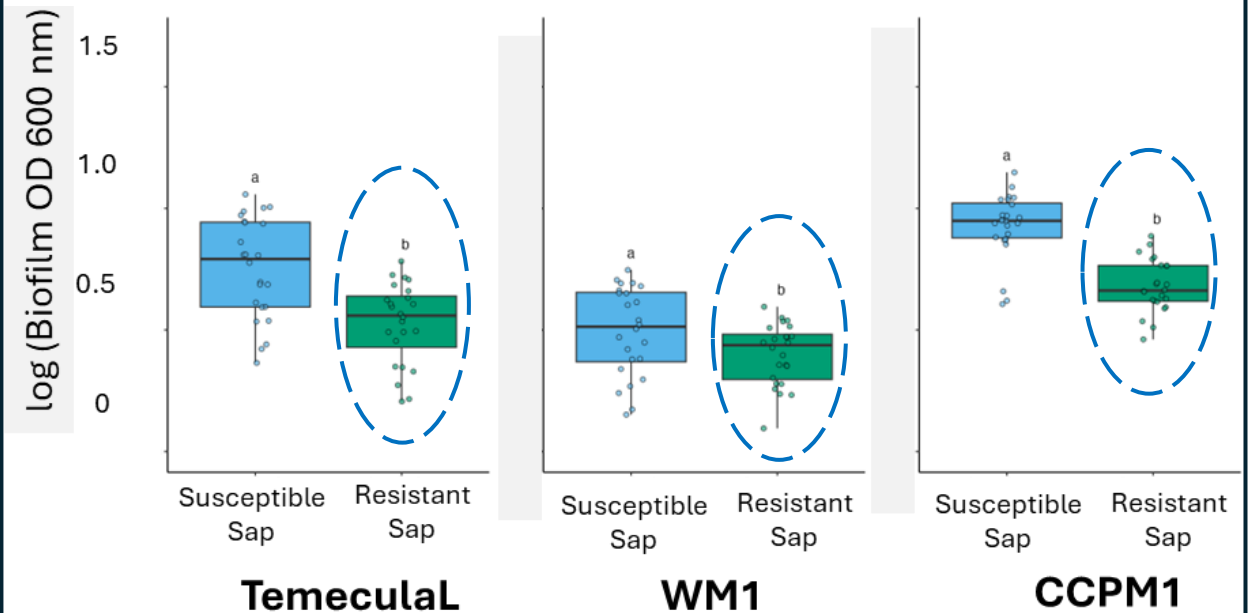
Susceptible Cultivar: Chardonnay

Resistant Cultivar: Blanc du Bois

Grape-Isolated Strains:
TemeculaL, WM1, CCPM1



Grapevine



 **Susceptible** sap 'Chardonnay'

 **Resistant** sap 'Blanc Du Bois'



Adaptation to sap during subcultures



Serial Passage Experiment design

WT strains

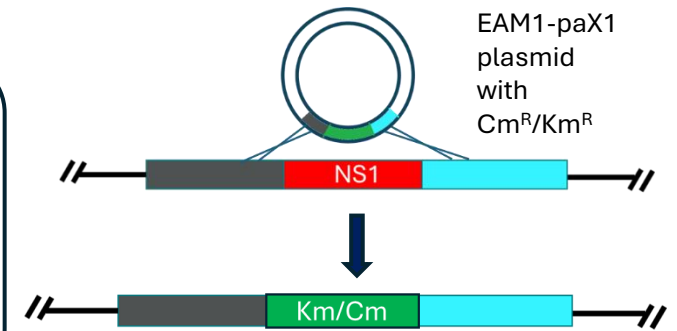
ALS17T10- High Biofilm, virulent

ALS6- Low Biofilm, avirulent

Antibiotic resistance

ALS17T10 :: Cm^R

ALS6 :: Km^R



Matsumoto et al., 2009

Treatments

ALS17T10^{CmR}

ALS6^{KmR}

ALS17T10^{CmR} + dead (ALS6)

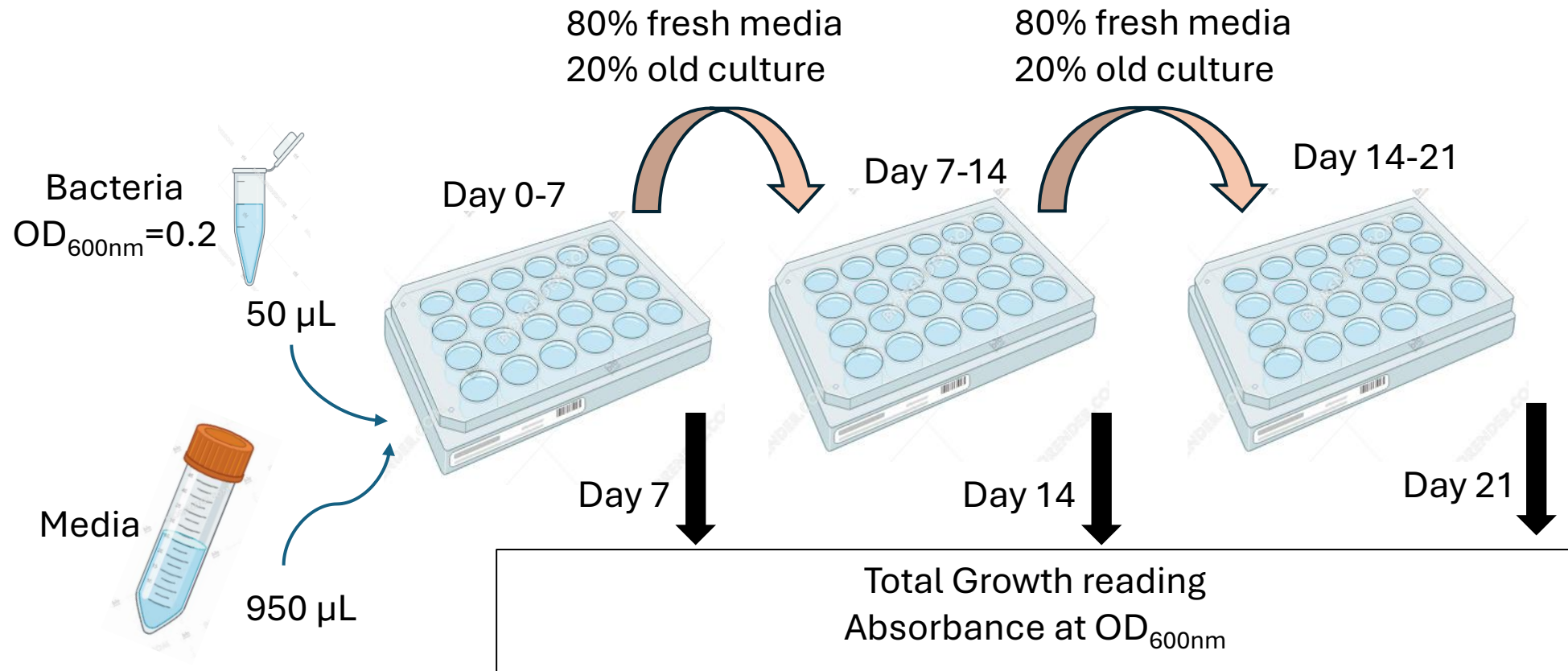
ALS6^{KmR} + dead (ALS17T10)

Media

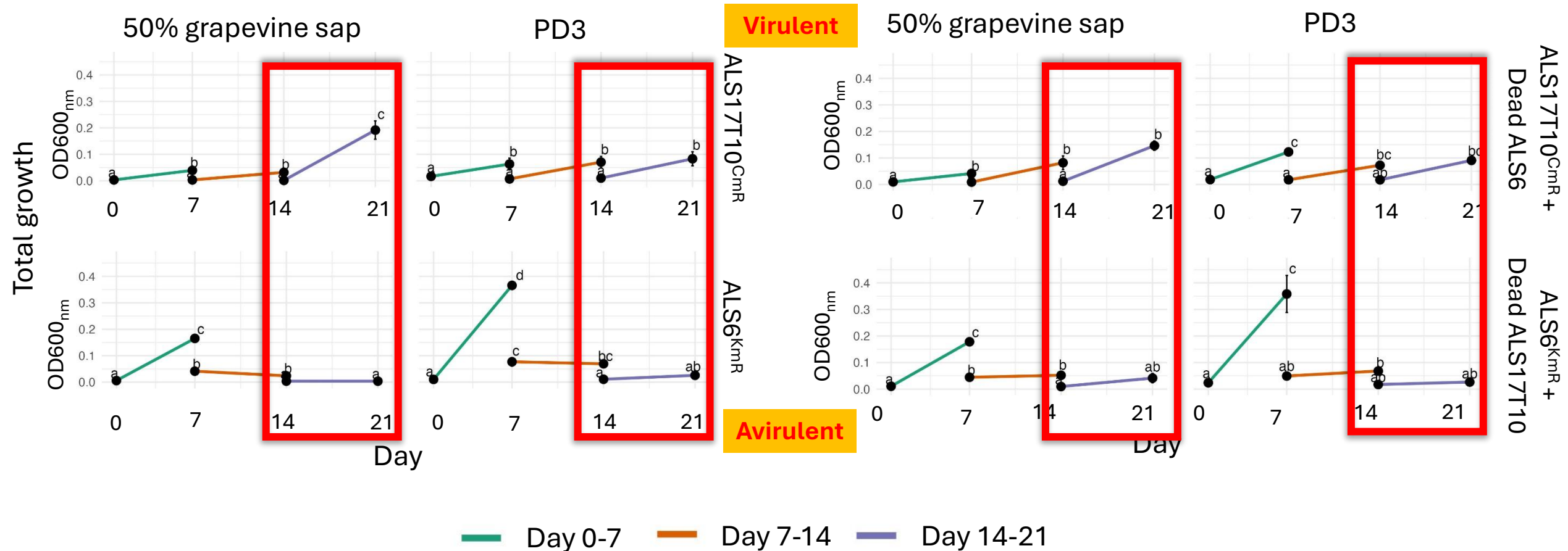
PD3

PD3+ 50% Grapevine sap
Susceptible cv 'Chardonnay'

Serial Passage Experimental setup



Serial Passage in grape sap vs. PD3



Virulent strain adapts better to grow in sap

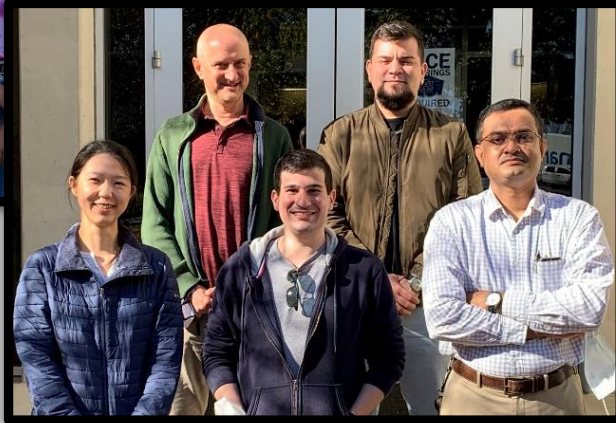
Conclusions

- **European *X. fastidiosa*** strains can cause symptoms and colonize low chill blueberry varieties.
- Most (70-85%) of the strains tested in vitro showed that **increase in biofilm** formation with **sap amendments** corresponds to **virulence** in that host.
- Selected strains form **less biofilm** when **tolerant sap** was added to media, as compared to susceptible sap
- Virulent strain **adapt** to grow better in sap after serial subcultures
- Hypothesis: **sap composition can be a determinant of infection**

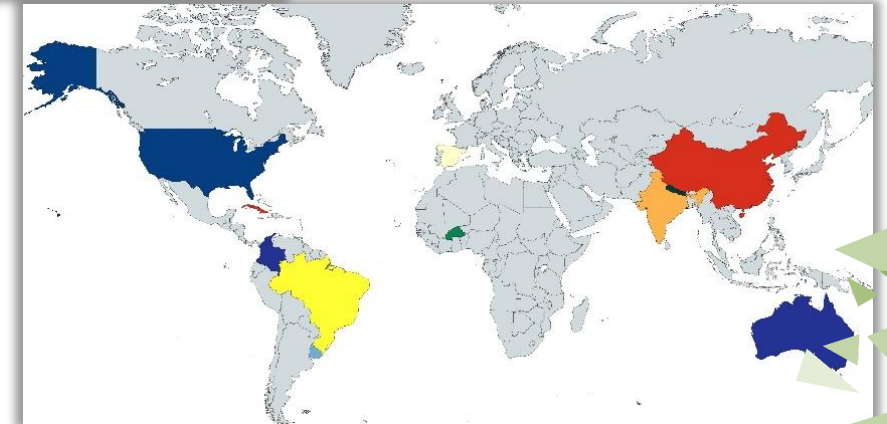
Acknowledgements

Authors: Mamata KC, Navdeep Kaur, Allison Belviy

Collaborators: Marcus Merfa, Alexandra Kahn, Rodrigo Almeida, Deepak Shantharaj



Funding:





Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026

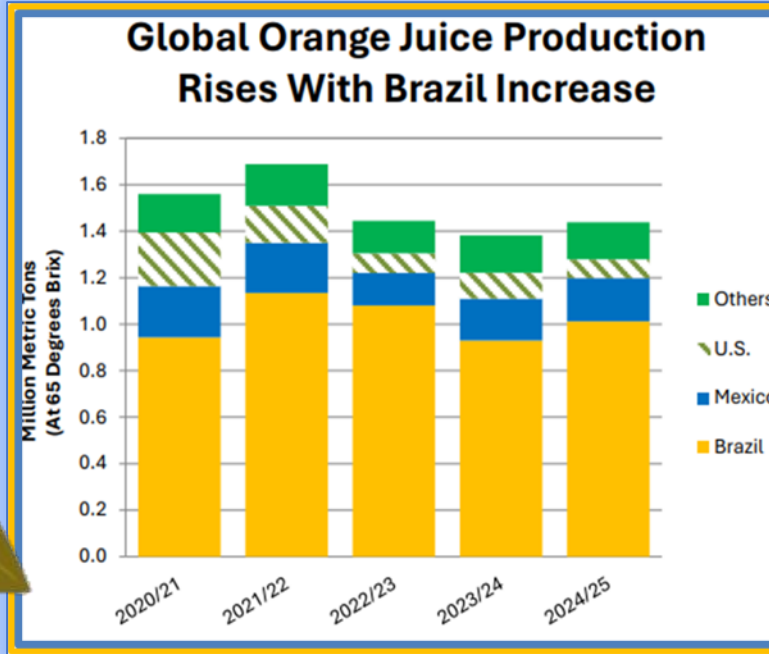
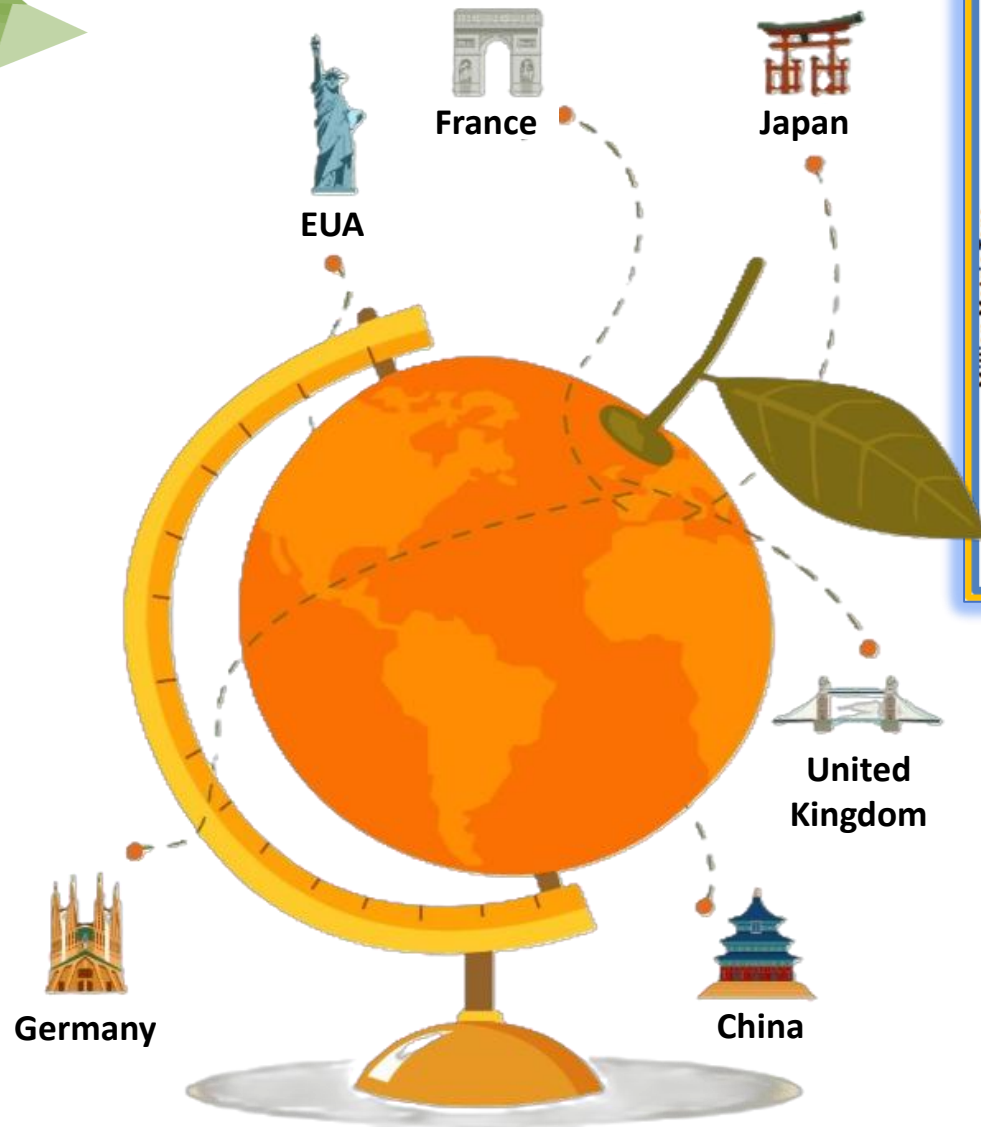


REGIONE PUGLIA

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

Victoria Stern da Silva
Citrus Research Center 'Sylvio Moreira'
Agronomic Institute - IAC
University of Campinas - UNICAMP
victoria.agro58@gmail.com

Global Orange production



Brazil is the world's largest producer of sweet oranges and the largest exporter of orange juice



Brazil accounts for approximately 80% of the orange juice consumed worldwide.



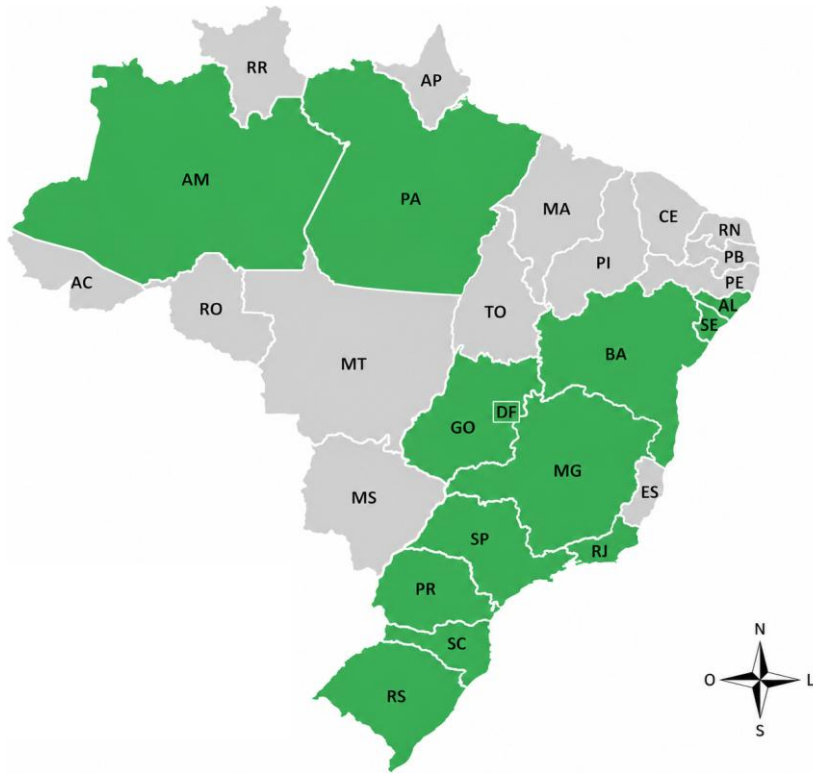
Citrus Variegated Chlorosis (CVC)

Xylella fastidiosa



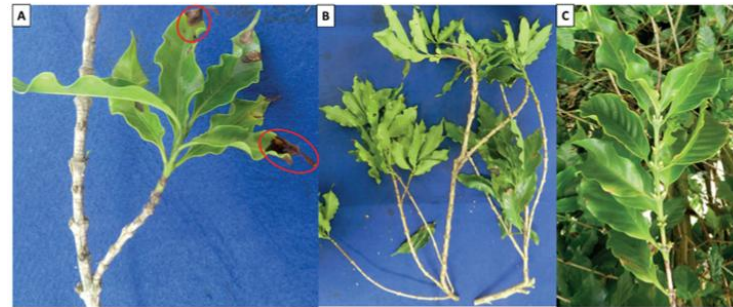
Xylella fastidiosa is a multi-host pathogen

Brazilian States with CVC occurrence



Plum leaf scald

Main factor for the crop decline in the southern and southeastern states of the country



Coffee leaf scorch disease

CVC is reported in several brazilian states

Olive quick decline syndrome



X. fastidiosa quorum sensing (QS)

X. fastidiosa QS is regulated by diffusible signal factor (DSF) molecules

Disease severity is associated with bacterial movement through the xylem



Bacterial movement within the xylem is regulated by quorum sensing

Xylem sap



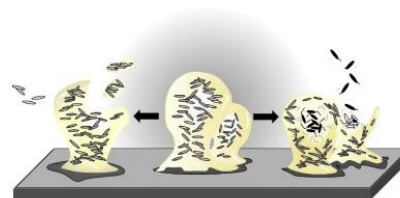
↑ Genes that code for

Biofilm – Sessile

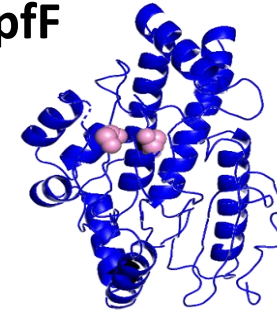
Afimbrial
Adhesins

Type I pili

EPS synthesis



RpfF



↓ Genes that code for

Planktonic - Movement

Type VI pili

Cell Wall degradation
enzymes (exoenzymes)

DSF



Conserved short-
chain fatty acids

Control the expression
of genes according to
population density

DSF beyond a quorum sensing molecule

Could plants expressing *rpfF* affect
bacterial signaling, preventing the
bacteria movement?

X. fastidiosa quorum sensing (QS)



Transgenic plants expressing the *rpfF* gene

Ectopic Expression of *Xylella fastidiosa* *rpfF* Conferring Production of Diffusible Signal Factor in Transgenic Tobacco and Citrus Alters Pathogen Behavior and Reduces Disease Severity

R Caserta¹, R R Souza-Neto^{1 2}, M A Takita¹, S E Lindow³, A A De Souza¹

Production of *Xylella fastidiosa* diffusible signal factor in transgenic grape causes pathogen confusion and reduction in severity of Pierce's disease

Steven Lindow, Karyn Newman, Subhadeep Chatterjee, Clelia Baccari, Anthony T Lavarone, Michael Ionescu

PMID: 24499029 DOI: [10.1094/MPMI-07-13-0197-FI](https://doi.org/10.1094/MPMI-07-13-0197-FI)



rpfF

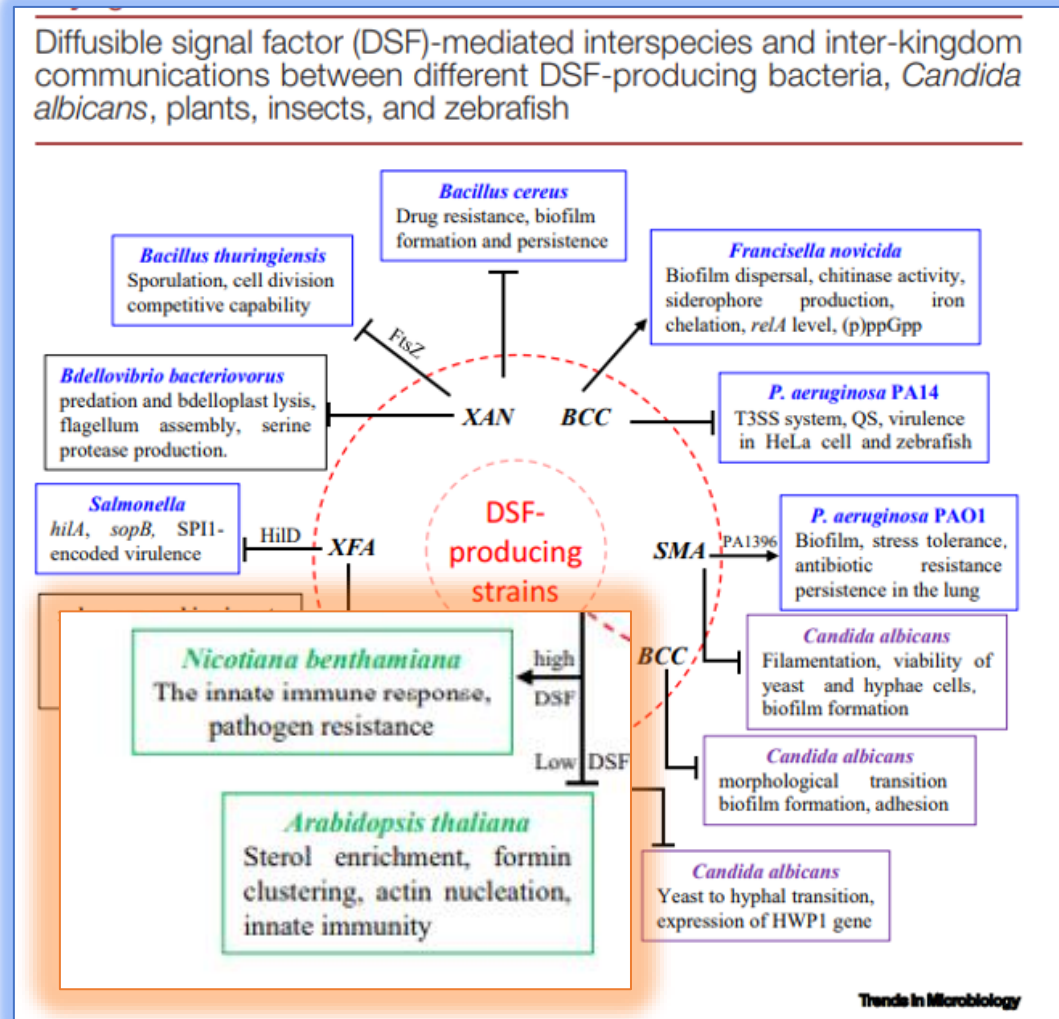


Pathogen confusion strategy

Increased expression of genes conferring adhesiveness to the pathogen and decreased expression of genes related to motility

DSF beyond a quorum sensing molecule

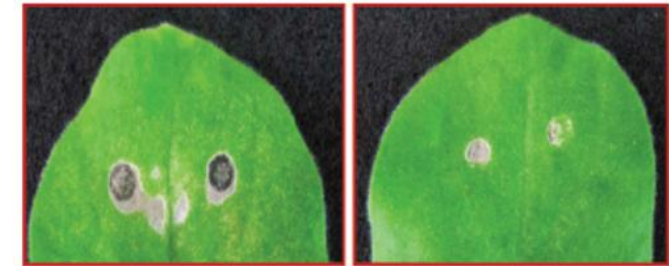
DSF mediates inter-Kingdom communications



> J Exp Bot. 2015 Nov;66(21):6697-714. doi: 10.1093/jxb/erv377. Epub 2015 Aug 5.

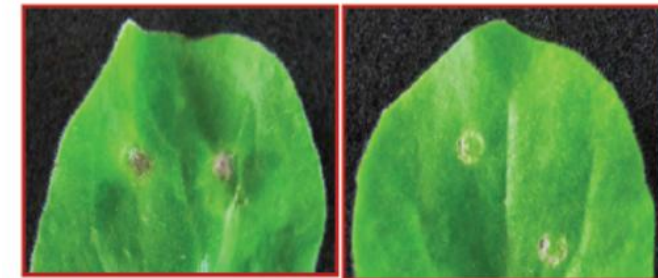
Xanthomonas campestris cell-cell signalling molecule DSF (diffusible signal factor) elicits innate immunity in plants and is suppressed by the exopolysaccharide xanthan

Akanksha Kakkar ¹, Narasimha Rao Nizampatnam ², Anil Kondreddy ², Binod Bihari Pradhan ², Subhadeep Chatterjee ³



1mM DSF

0.5mM DSF



0.25mM DSF

0.1mM DSF

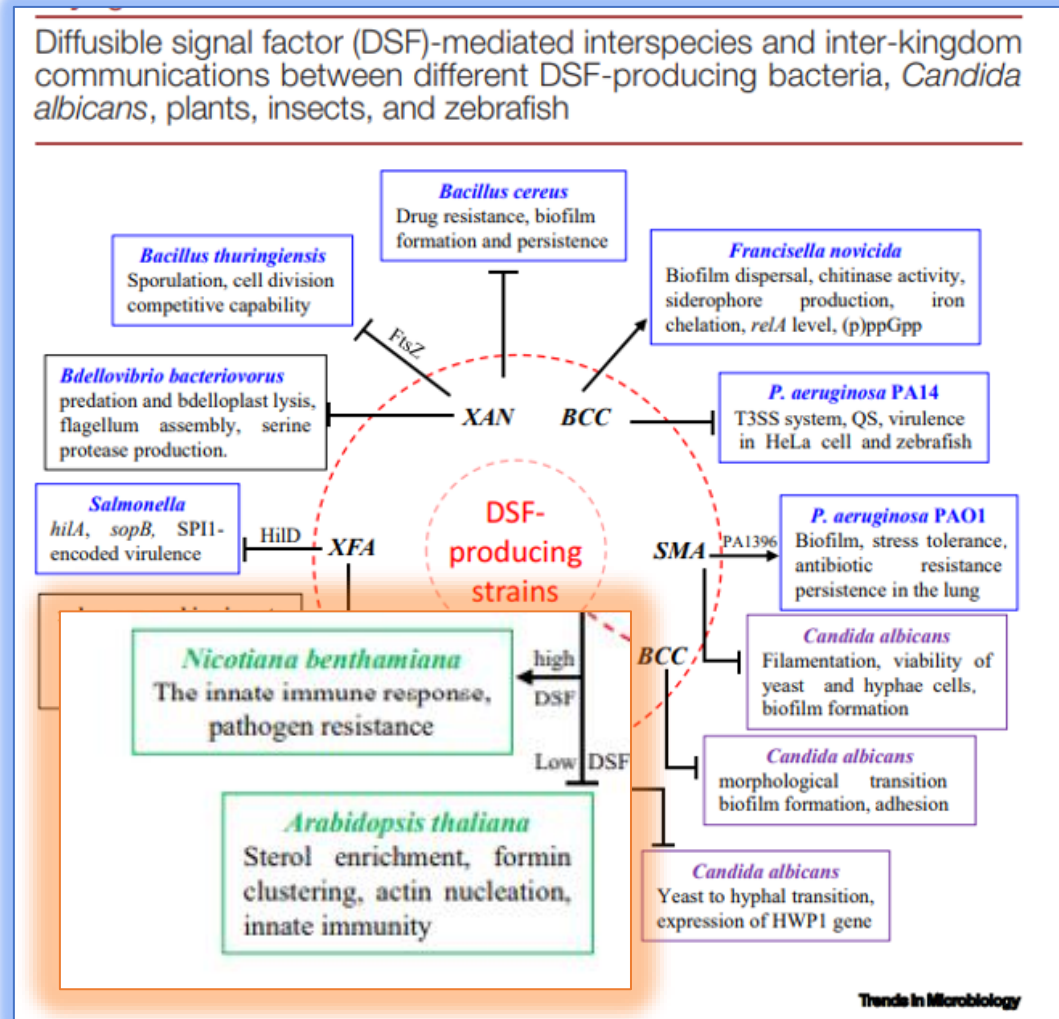


Control

DSF from *Xanthomonas campestris* triggers HR

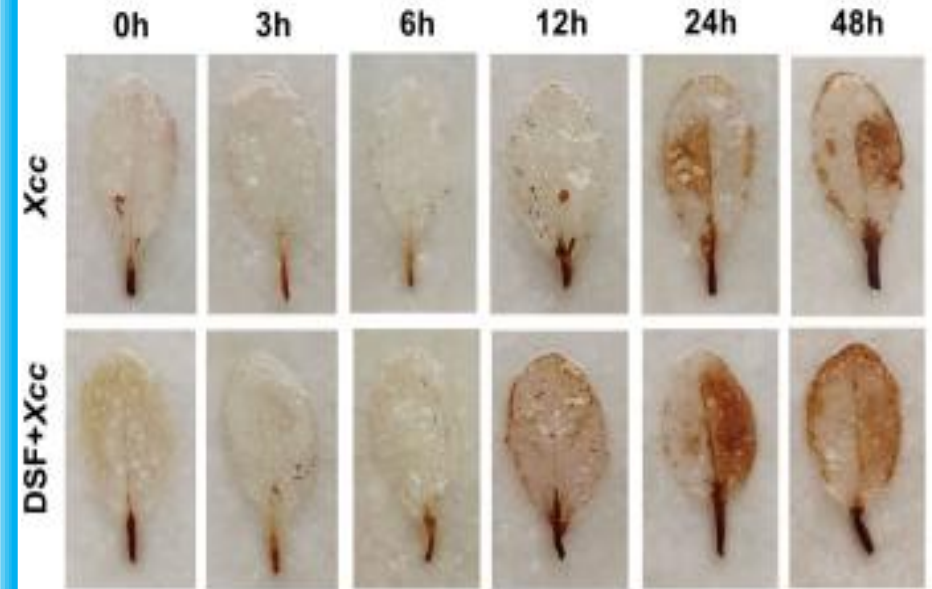
DSF beyond a quorum sensing molecule

DSF mediated inter-Kingdom communications



Diffusible signal factor primes plant immunity against *Xanthomonas campestris* pv. *campestris* (Xcc) via JA signaling in *Arabidopsis* and *Brassica oleracea*

Front. Cell. Infect. Microbiol., 19 June 2023
Sec. Molecular Bacterial Pathogenesis
Volume 13 - 2023 |
<https://doi.org/10.3389/fcimb.2023.1203582>



Pretreatment with DSF and subsequent pathogen invasion triggered an increased burst of ROS

Infiltration of DSF primed plant immunity against Xcc in both *Brassica oleracea* and *Arabidopsis thaliana*.

DSF beyond a quorum sensing molecule

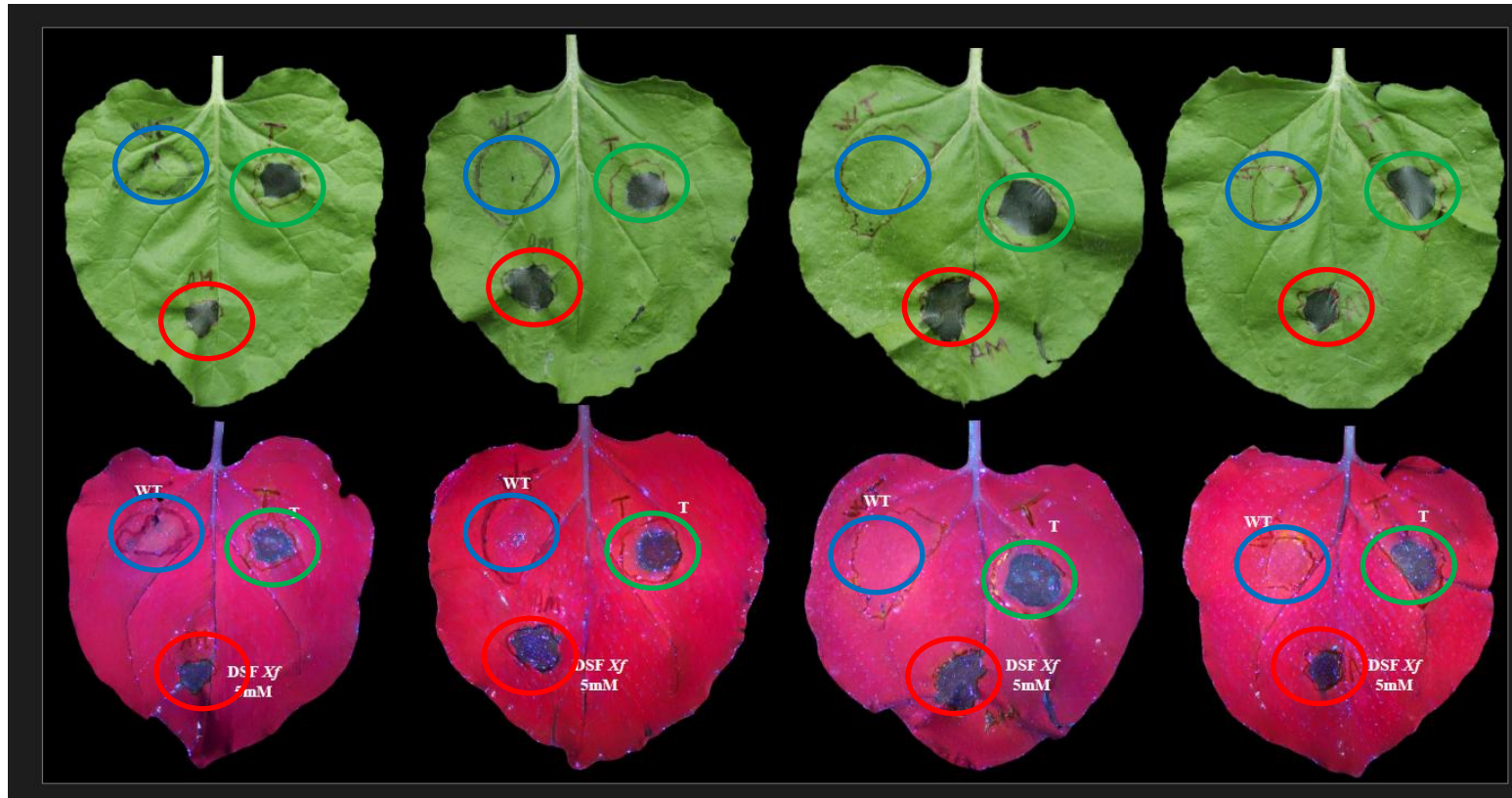
Could DSF also act as an elicitor molecule in transgenic citrus plants?

Detection of hypersensitivity reactions (HR)

1°

Hypersensitivity reactions (HR)

To investigate whether plants can recognize DSF



HR was only detected at the sites where synthetic DSF and the extract from transgenic plants were applied

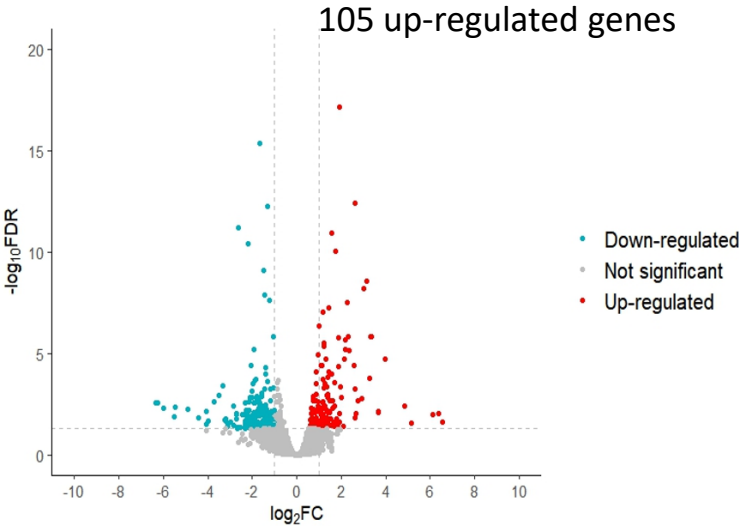
The induction of a hypersensitive response suggests that DSF is perceived by the plant

RNA-seq analysis

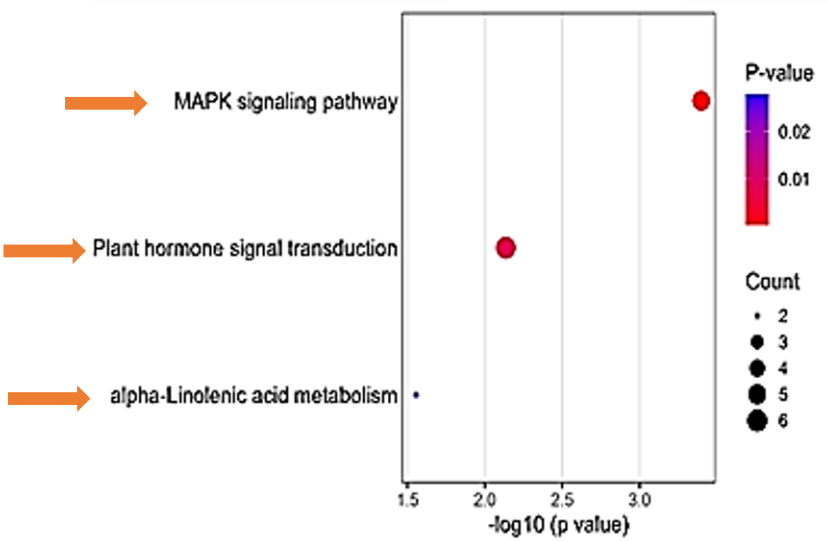
2°

Transcriptome

To determine whether DSF elicits plant immunity



KEGG enrichment analysis



Oxidative stress-response	Lipid metabolism	ET/JA responsive TF	PR-proteins
<i>PRX</i> <i>SOD</i> <i>GalS</i> <i>GST</i>	<i>HPL</i> <i>LOX2</i> <i>CAS</i> <i>PLD</i>	<i>FAR1</i> <i>AP2/ERF</i>	<i>KTI</i> <i>ITH</i> <i>CHIB</i>



Mass Spectrometry-Based Targeted Lipidomics and Supervised Machine Learning Algorithms in Detecting Disease, Cultivar, and Treatment Biomarkers in *Xylella fastidiosa* subsp. *pauca*-Infected Olive Trees

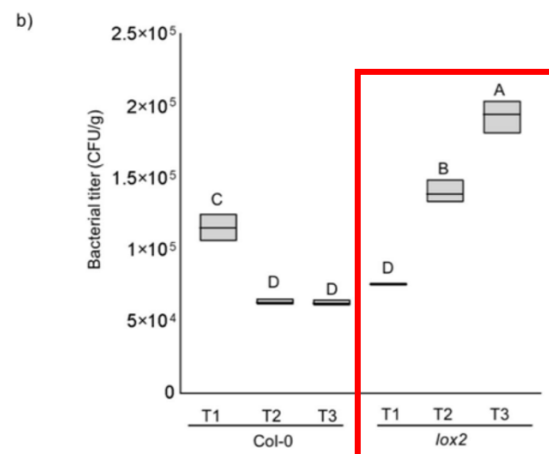
Valeria Scala^{1*}, Manuel Salustri², Stefania Loreti¹, Nicoletta Pucci¹, Andrea Cacciotti², Giuseppe Tatulli¹, Marco Scortichini³ and Massimo Reverberi²



Article

Assessment of Fatty Acid and Oxylipin Profile of Resprouting Olive Trees Positive to *Xylella fastidiosa* subsp. *pauca* in Salento (Apulia, Italy)

*¹ , Marco Scortichini², Federico Marini³ , Dario La Montagna⁴ , Marzia Beccaccioli⁴ , izzi⁴, Andrea Cacciotti⁴ , Nicoletta Pucci¹ , Giuseppe Tatulli¹ , Riccardo Fiorani¹, i¹ and Massimo Reverberi⁴



The oxylipin profile is important for defense against *Xylella*

HPL → Hydroperoxide lyase

LOX2 → Lipoxygenase 2

CAS

PLD

Linoleic acid

LOX2

HPL

Fatty acid hydroperoxides

Volatile oxylipins GLVs

Fatty acid hydroperoxides

JA

AOS

The reduction of 13-oxylipins allows the pathogen to move and colonize the host to a greater extent

Gramegna et al. Discover Plants (2025) 2:233
<https://doi.org/10.1007/s44372-025-00320-2>

Discover Plants

<https://doi.org/10.1007/s44372-025-00320-2>

RESEARCH

Open Access



AtLOX2 plays a role in contrasting the growth of *Xylella fastidiosa* subsp. *fastidiosa* Temecula1 in the model *Arabidopsis thaliana*

G. Gramegna¹, M. Beccaccioli¹, N. Pucci², S. Loreti², M. Reverberi¹ and V. Scala^{2*}

Lipidome analysis

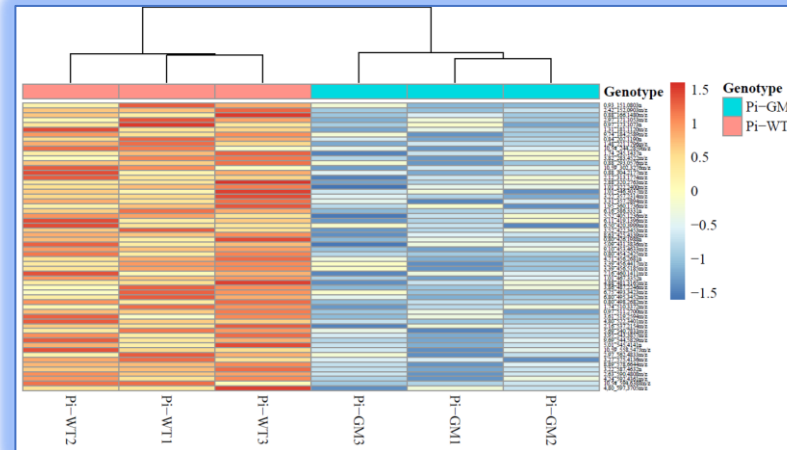
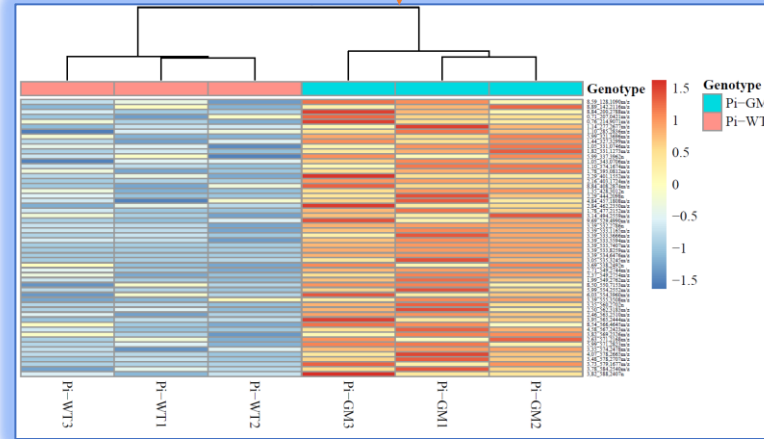
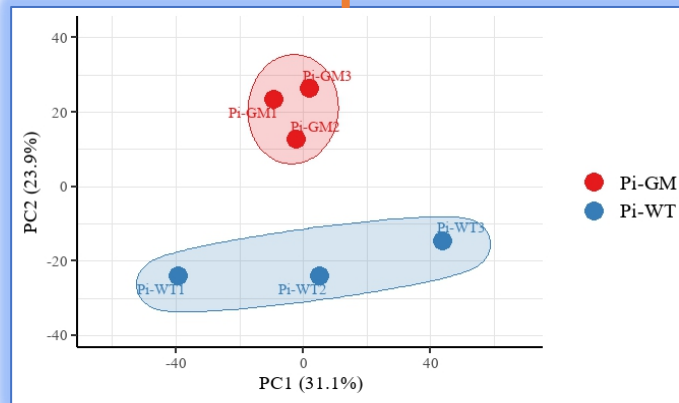
3°

Alteration in the fatty acid profile

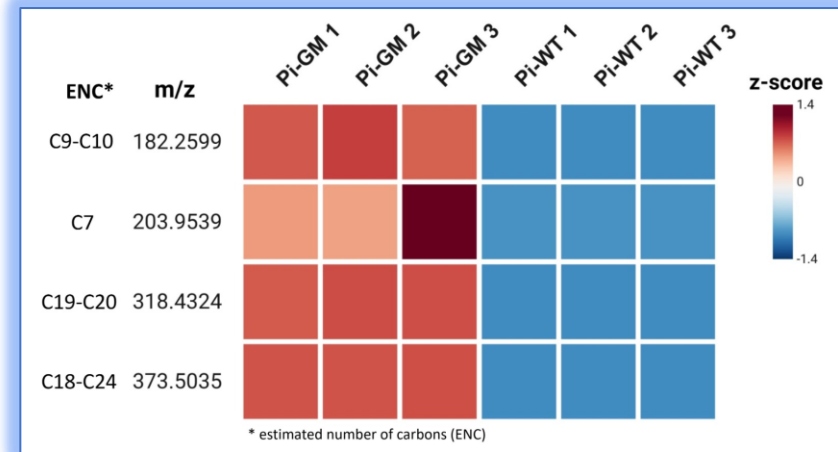
The RpfF enzyme is involved in fatty acid biosynthesis

Lipidome

Identify the profile of fatty acids in the transgenic plants in relation to the WT



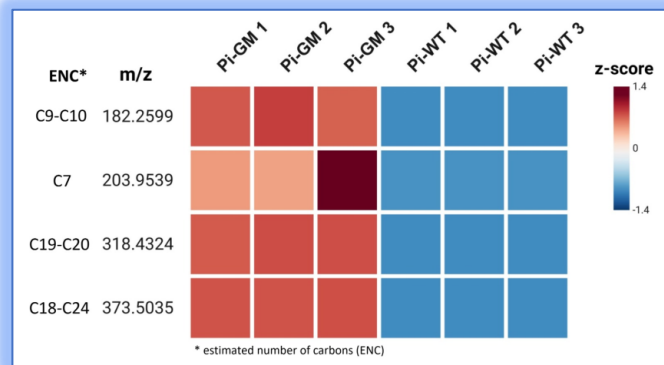
Fatty acids detected exclusively in transgenic plants



Lipidome analysis

3°

Fatty acids detected exclusively in transgenic plants

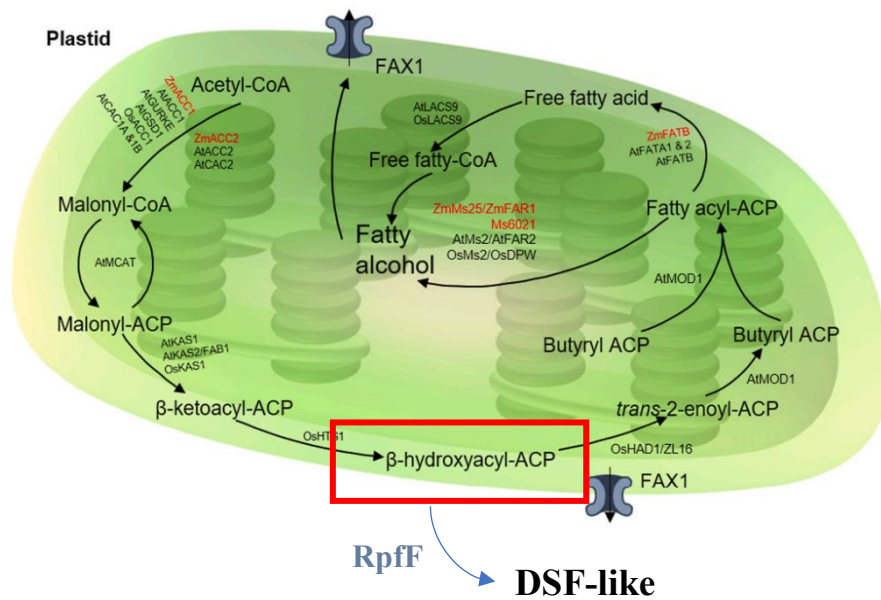


RpfF activity

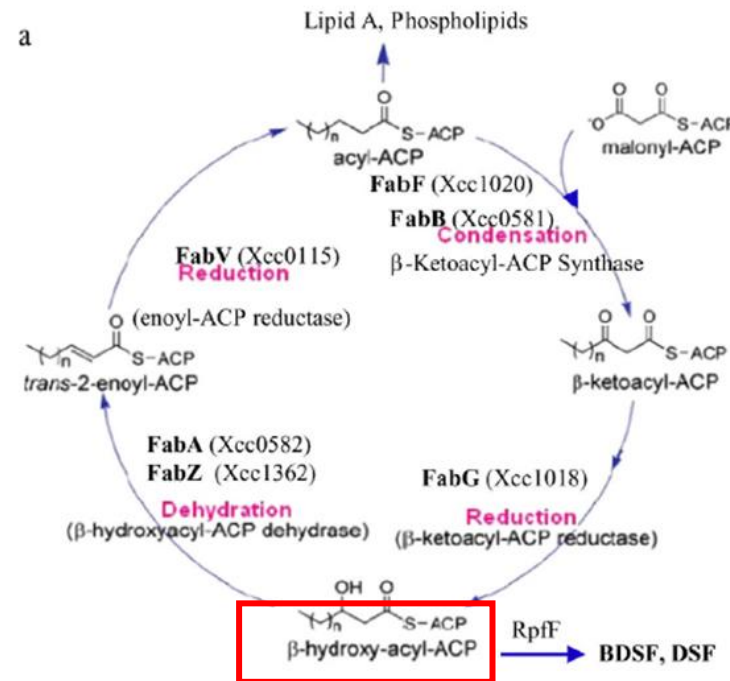
Lipidome

Identify the profile of fatty acids in the transgenic plants in relation to the WT

Fatty acid biosynthesis in plants



Fatty acid biosynthesis in bacteria



Lipidome analysis

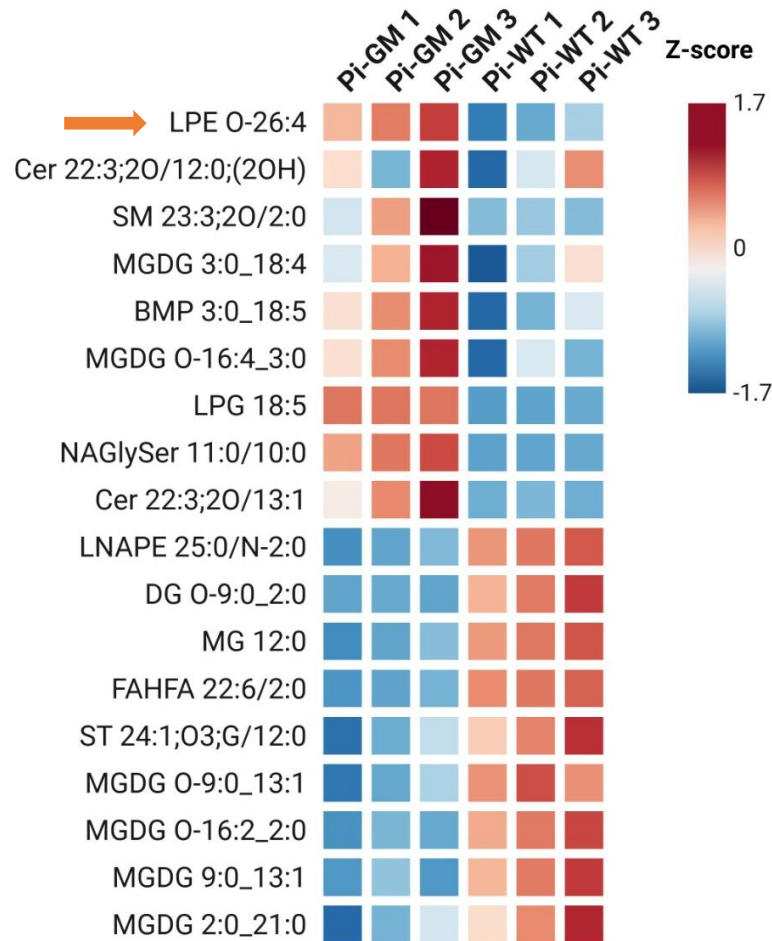
Lipidome

3°

Identify the profile of fatty acids in the transgenic plants in relation to the WT

The RpfF enzyme is involved in fatty acid biosynthesis

Alteration in the lipid profile



LPE Primes Plant immune system

Lyso-phosphatidylethanolamine triggers immunity against necrotrophs by promoting JA-signaling and ROS-homeostasis

[Ronny Völz](#)^{1,✉}, [Ki-Tae Kim](#)², [Mazen Alazem](#)³, [William Harris](#)⁴, [Sungkee Hwang](#)⁵, [Yong-Hwan Lee](#)^{1,4,6,7,8,✉}

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PMCID: PMC10721665 PMID: [38085407](#)

DOI: 10.1007/s11103-023-01385-x

Hypothetical Model of CVC Resistance in Transgenic Citrus Plants

Healthy plants

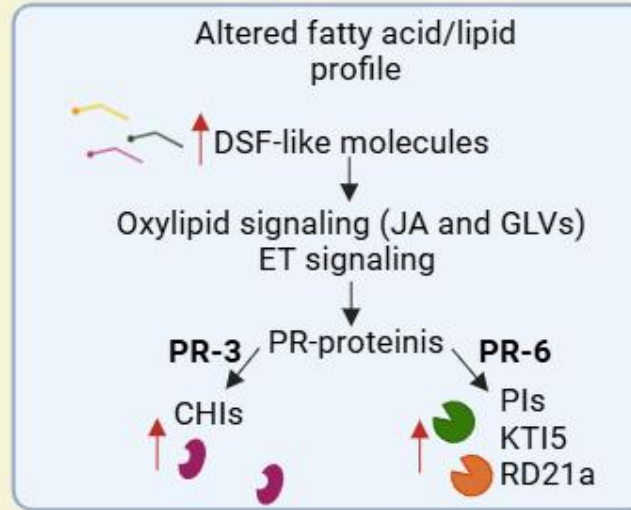
rpfF-transgenic plants



non-transgenic plants



(a)



Pathogen confusion
+
Priming effect

(b)



Susceptible
genotype



THANK YOU!

Alessandra Alves de Souza

Mariana de Souza e Silva

Mariana Bossi Esteves

Maíra Rossi

Daniela Beatriz Silva

Paulo Victor Maciel

Vinicius Ferreira

Lucas Nascimento

Matheus Armelin Nogueira

Flávia Bento

Daniela Feltrin

Giovanni Santiago



*Xylella
fastidiosa*

5th European conference

Bari, Italy | 22-26 June 2026

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

Carlos Selles-Ribera, Pilar Domingo-Calap, Ester Marco-Noales

Bacteriophages & Phage-based biocontrol

Bacteriophages are viruses that specifically infect and kill bacteria

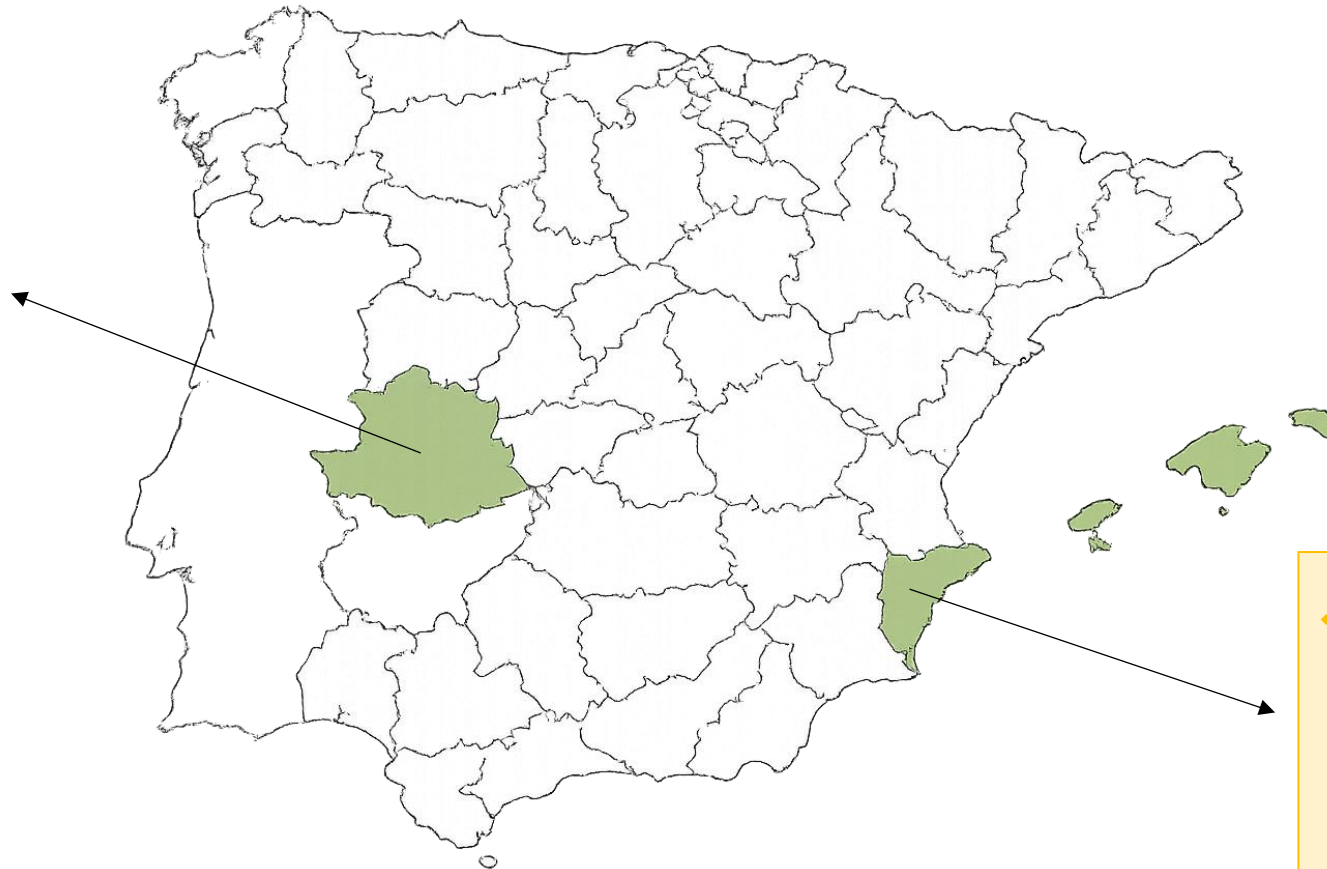
Phage-based biocontrol exploits these natural predators to selectively reduce harmful bacterial populations



Active mainland outbreaks in Spain

❖ *Xf* subsp. *fastidiosa* ST1

- *Vitis vinifera*
- *Cystus* sp.
- *Lavandula* sp.
- *Ulex* sp.
- ...



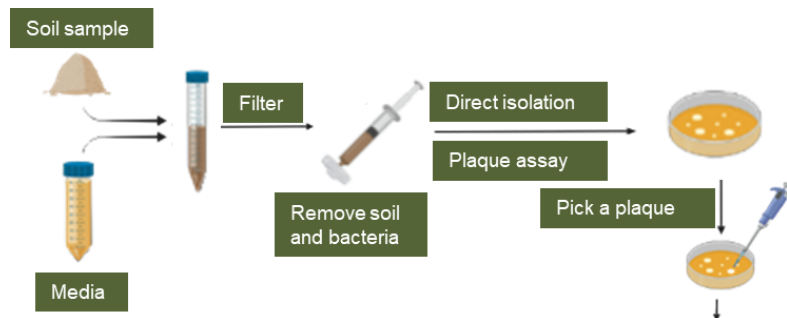
❖ *Xf* subsp. *multiplex* ST6

- 30 species
(90% of detections in *Prunus dulcis*)

Strategies towards *Xf* phage isolation

Surrogate host isolation

- ✓ Faster screening process
- ✓ Lower biosafety requirements
- ✓ Suitable production host available
- ✗ Uncertain success rate against the target



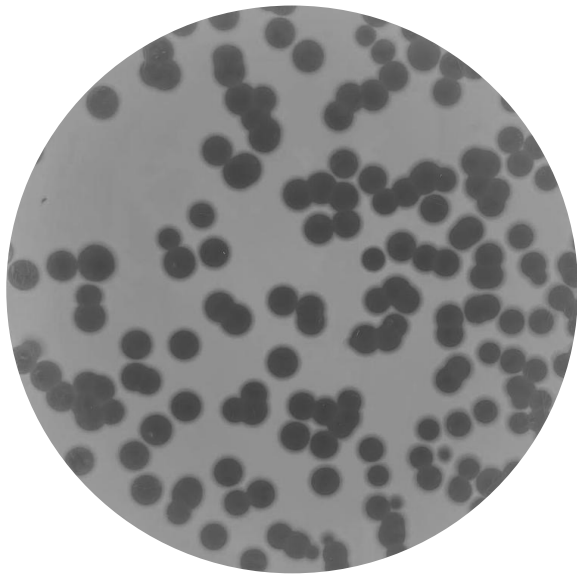
Direct target isolation

- ✓ Directly targets the pathogen
- ✓ Greater chance of finding strain-specific phages
- ✓ Better understanding of phage diversity
- ✗ Slower and more labor-intensive
- ✗ Highly specific phages may be difficult to propagate at large-scale for agricultural applications

Strategies towards *Xf* phage isolation

Surrogate host isolation

Direct target isolation



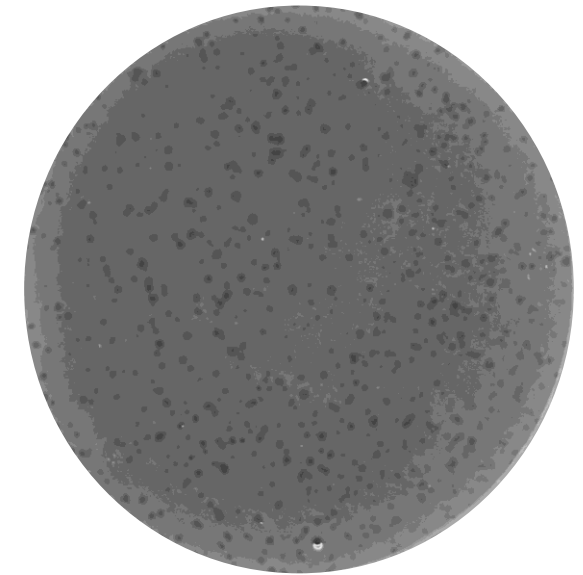
ViSe_6 phage on a *Xanthomonas arboricola* overlay

Xf bacteriophage
collection

25 phages

17 phages

Both methods worked for
subsp. *fastidiosa*, but
only direct isolation
succeeded for subsp.
multiplex



Mir_2 phage on a *Xf* subsp. *multiplex* overlay

Xf phage diversity

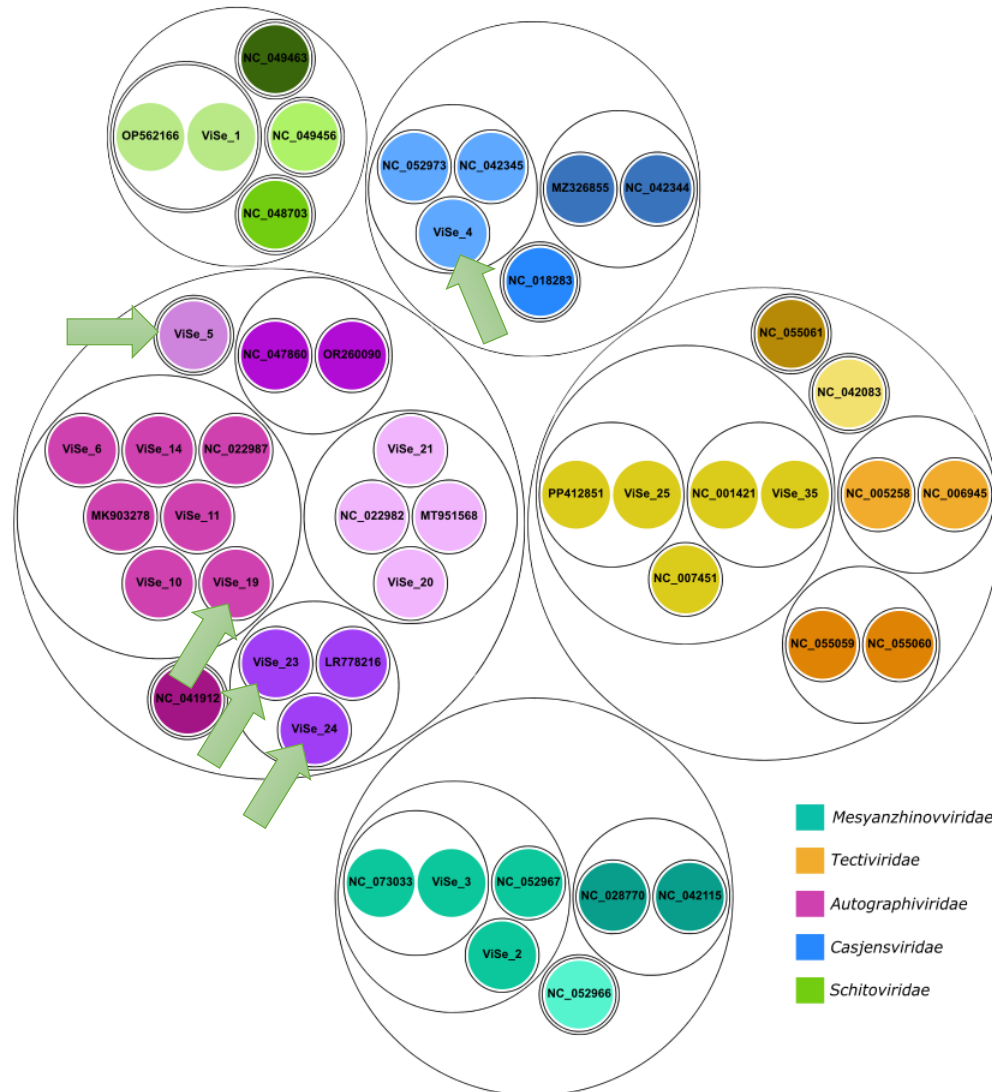


Phages isolated for subsp. *fastidiosa* exhibited high genomic diversity



Four novel genera and nine new species were proposed

Xf phage diversity



Phages isolated for subsp. *fastidiosa* exhibited high genomic diversity

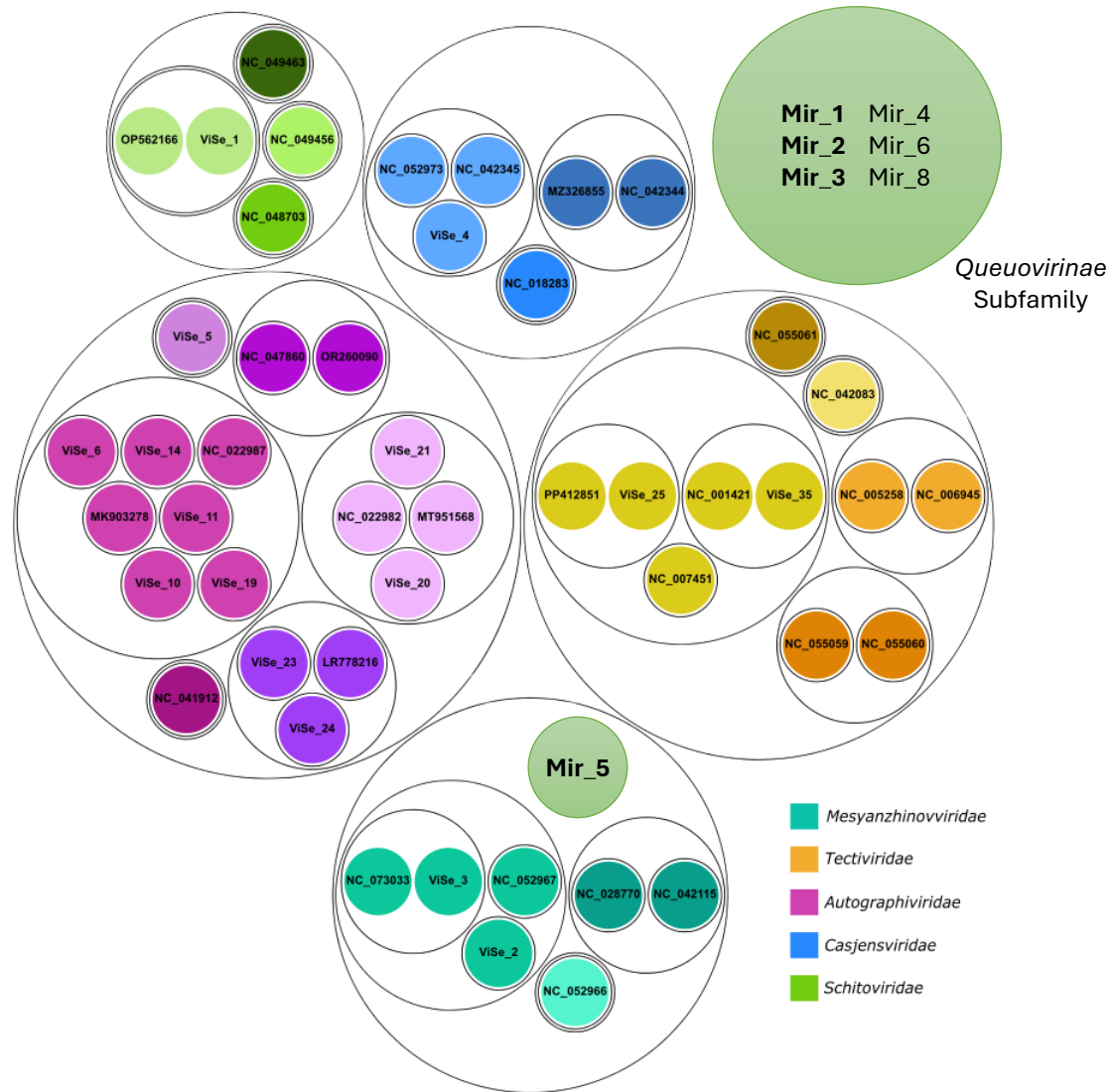


Four novel genera and nine new species were proposed

Two genera and five species were found only by direct isolation on *Xf*

For most of them, no suitable surrogate host within the genus *Xanthomonas* has been found for these phages

Xf phage diversity





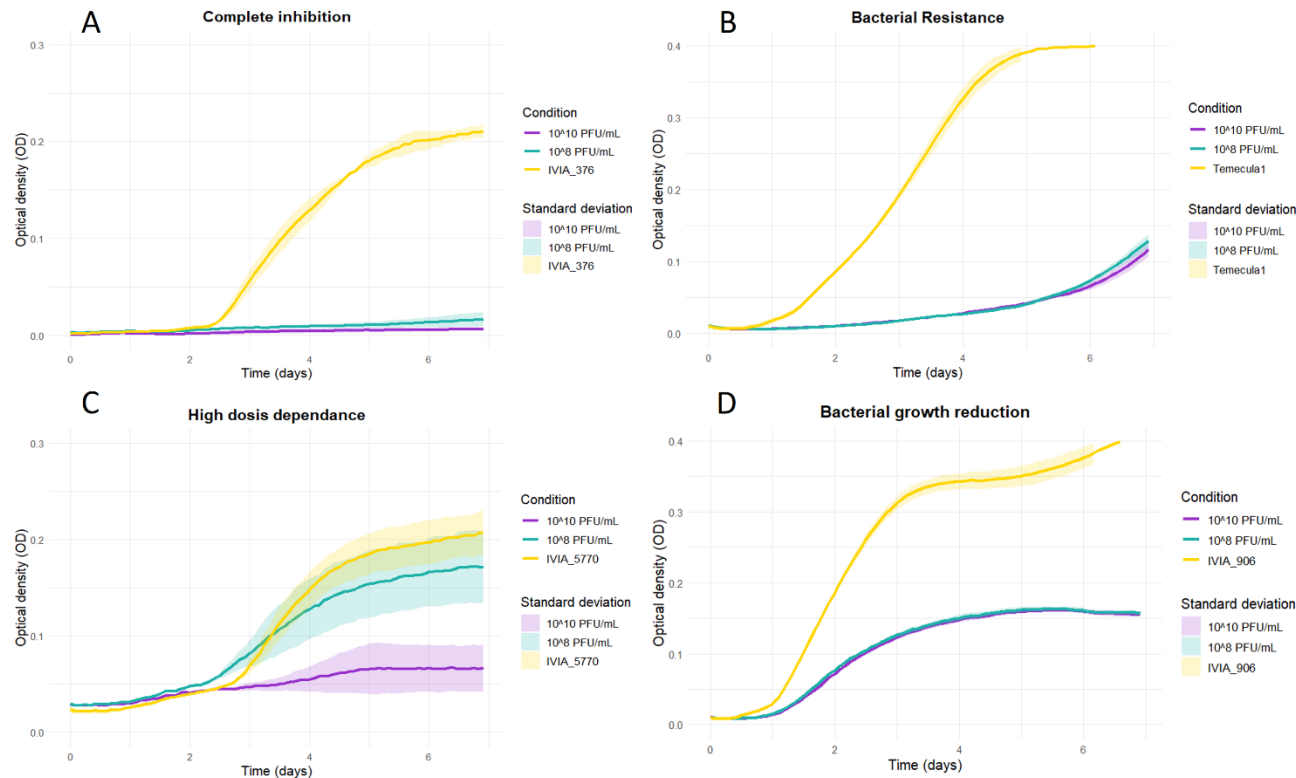
Choosing the right *Xf* phage isolation strategy

The optimal isolation strategy depends on the final objective

- ❖ For studies based on ecology, phage diversity, phage-host interaction, strain typing, or phage-based monitoring, surrogate-host isolation is not ideal. By selecting only phages capable of infecting the surrogate host, this approach biases the recovered population toward broader host-range phages.
 - ❖ For biocontrol of *Xf*, both approaches are valid and complementary. However, large-scale phage production will ultimately require a suitable production host, whether identified before or after phage isolation.
- 

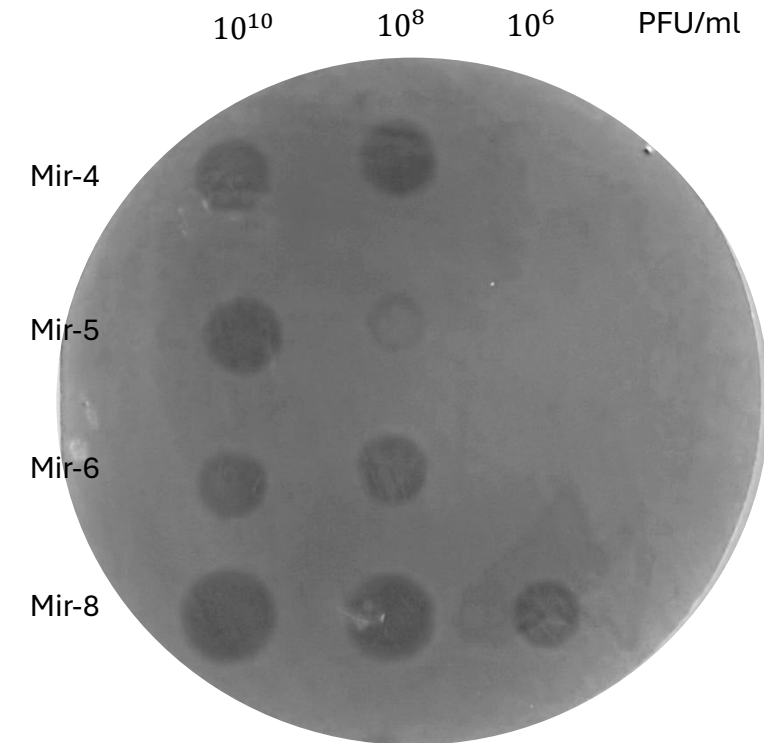
In vitro assays

Phage–bacteria dynamics, dose dependency and host range



Growth curves of *Xf* subsp. *fastidiosa* strains in the presence and absence of phages

Selles-Ribera *et al.* 2026. Plant Dis :[10.1094/PDIS-09-25-1903-RE](https://doi.org/10.1094/PDIS-09-25-1903-RE)



Spot assay with serial dilutions of ST6-isolated phages on a lawn of strain ST51

In planta assays

Greenhouse trials under
controlled conditions



❖ *Xf* ST6 → *Prunus dulcis*

❖ *Xf* ST1 → *Prunus dulcis*
Vitis vinifera

Field trials in high-
incidence plots



*Xylella
fastidiosa*

5th European conference

Bari, Italy | 22-26 June 2026

Thank you for your attention



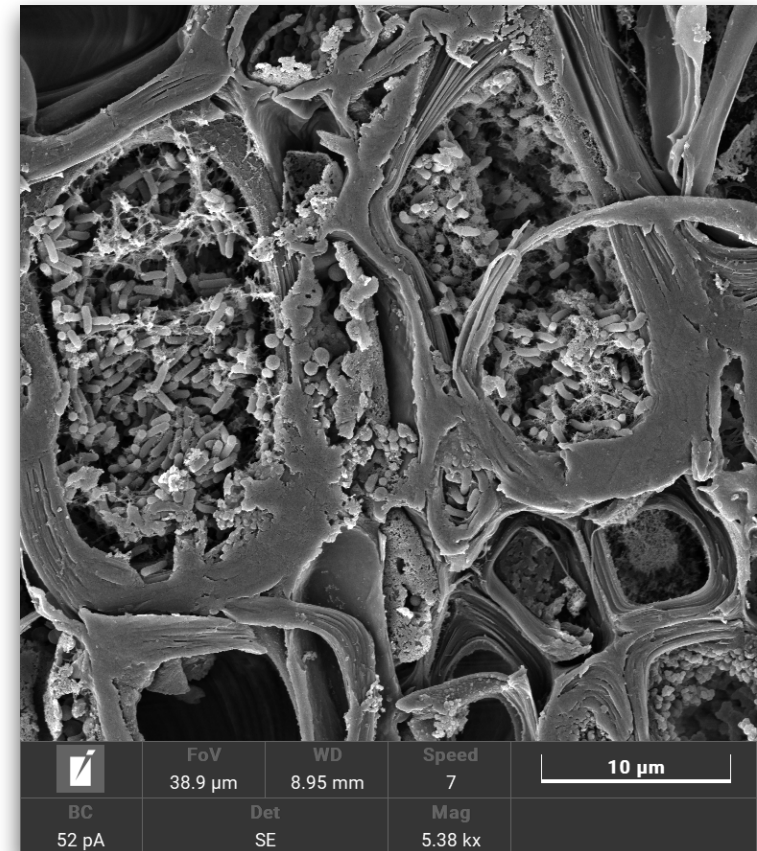
Overwinter recovery from Pierce's disease of grapevines

Ofir Bahar

Department of Plant Pathology and Weed Research
Agricultural Research Organization – Volcani Institute
ISRAEL

PIERCE'S DISEASE IN ISRAEL

- ▶ Pierce's disease (PD) is caused by *X. fastidiosa* subsp. *fastidiosa* (Xff)



PIERCE'S DISEASE IN ISRAEL

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- ▶ Xff was identified in northern Israel in 2017 in almonds



PIERCE'S DISEASE IN ISRAEL

- ▶ Pierce's disease (PD) is caused by *X. fastidiosa* subsp. *fastidiosa* (Xff)
- ▶ Xff was identified in northern Israel in 2017 in almonds
- ▶ And found in vineyards in the upper Galilee in 2021
- ▶ Xff (ST1)



PIERCE'S DISEASE IN ISRAEL

- ▶ Pierce's disease (PD) is caused by *X. fastidiosa* subsp. *fastidiosa* (Xff)
- ▶ Xff was identified in northern Israel in 2017 in almonds
- ▶ And found in vineyards in the upper Galilee in 2021
- ▶ Xff (ST1)
- ▶ Spreads primarily by *Mesoptelyelus impictifrons*

DAMAGE CAUSED BY PIERCE'S DISEASE (PD) TO THE WINE INDUSTRY IN CALIFORNIA

RESEARCH ARTICLE

Pierce's disease costs California \$104 million per year

by Kabir P. Tumber, Julian M. Alston and
Kate B. Fuller

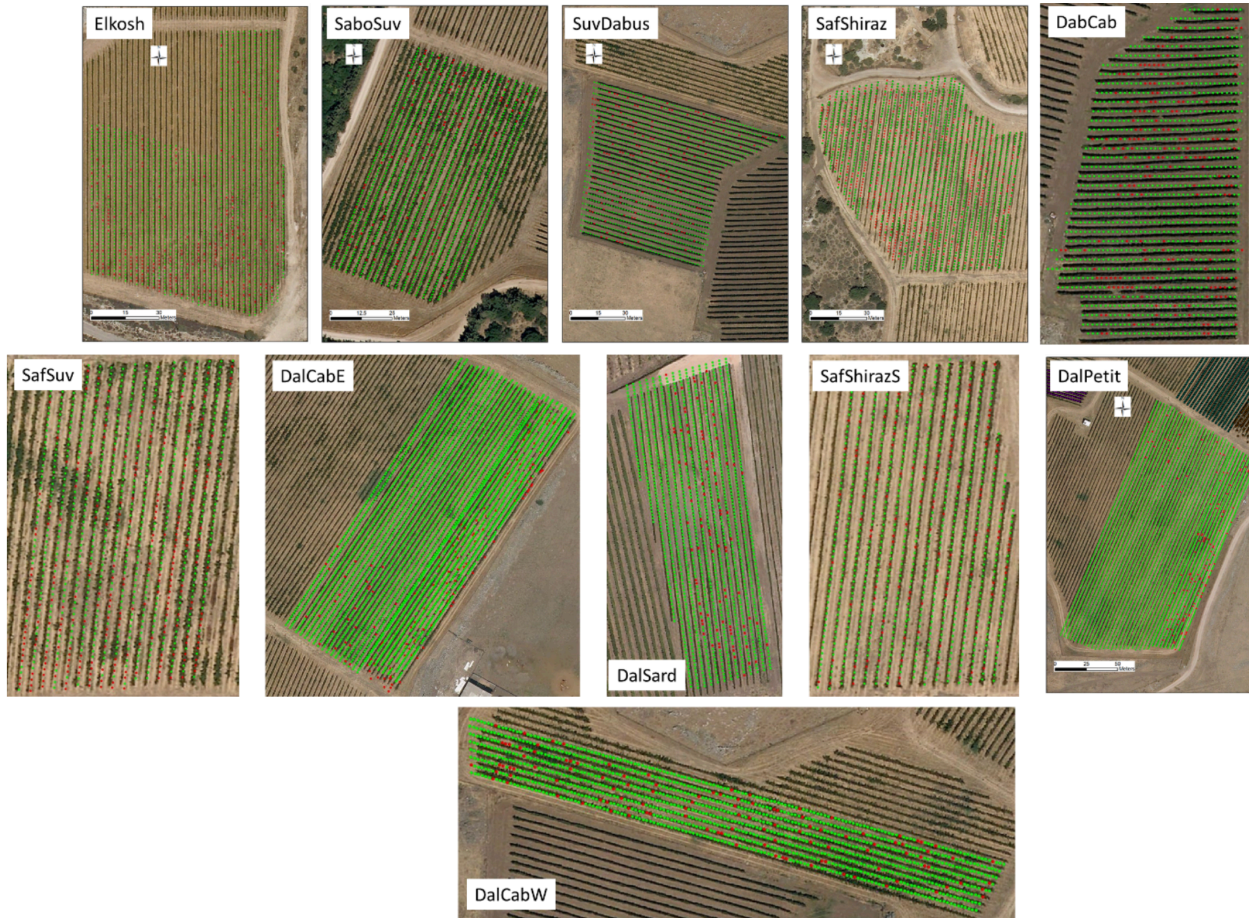
*Pierce's disease of grapevines, caused by a strain of the bacteria *Xylella fastidiosa*, threatens an industry with a farm value of production exceeding \$3 billion per year. The grape industry incurs substantial costs from losses of vines to the disease and efforts to mitigate damage. Additional costs are borne by the public in providing programs that aim to contain the disease and develop longer-term solutions, and by the citrus, nursery and grape industries in complying with those programs. Aggregating the costs of vine losses, industry assessments, compliance costs, and expenditures by government entities, we estimate the cost of Pierce's disease in California is approximately \$104.4 million per year. Of that, \$48.3 million funds Pierce's disease and the UC system, and \$56.1 million is the cost of lost production and vine replacement borne by grape growers.*



Jack Kelly Clark

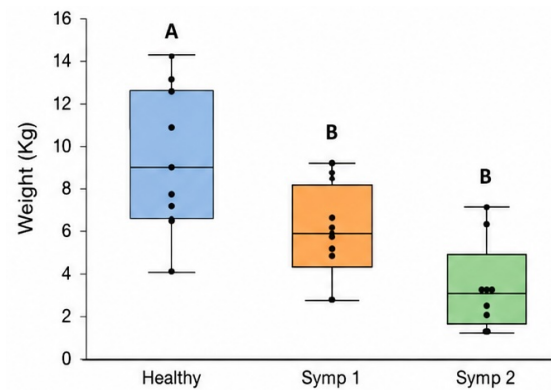
Since the late 1990s, tens of millions of dollars in public and private funding have been spent each year to prevent the spread and mitigate the effects of Pierce's disease in California.

PIERCE'S DISEASE IN ISRAEL

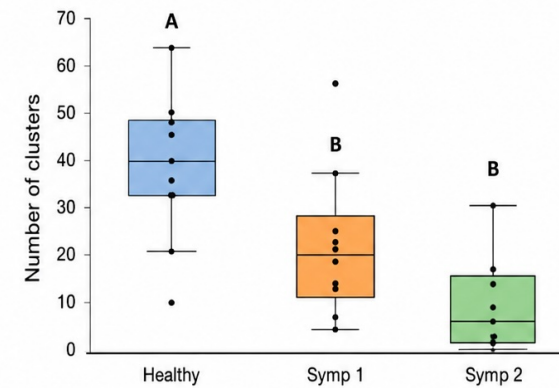
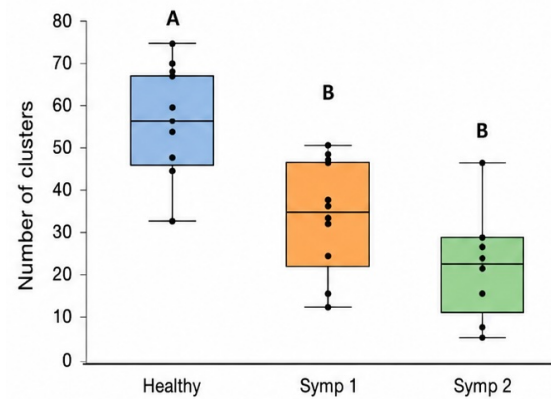
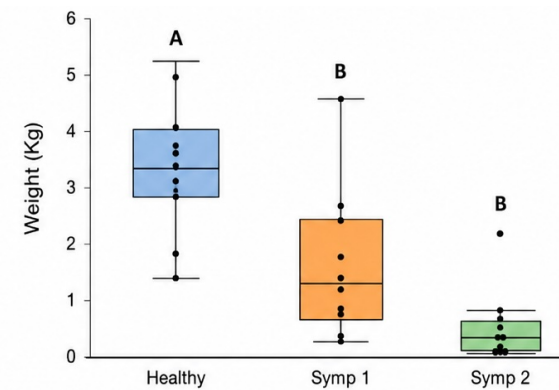


PIERCE'S DISEASE EFFECT ON YIELD

Sauvignon Blanc



Cabernet Sauvignon



OVERWINTER RECOVERY FROM PD IN NAPA VALLEY

- ▶ Elimination of pathogen and remission of symptoms
- ▶ PD overwinter recovery:
 - ▶ Reports from California
 - ▶ Deciduous plants
 - ▶ Associated with low winter temperatures



CAN OVERWINTER RECOVERY FROM PD OCCUR UNDER MEDITERRANEAN CONDITIONS AND WHAT ARE THE FACTORS AFFECTING IT?

- ▶ Geographical location, temperature
- ▶ Time of inoculation
- ▶ Cultivar

RECOVERY EXPERIMENT

- ▶ Cabernet Sauvignon and Chardonnay plants were inoculated with Xff (ST1) using the needle-prick method at 4 different time points from May to September.
- ▶ At each time-point 50 plants from each variety were inoculated
- ▶ Symptom and infection were assessed throughout the season
- ▶ When entering dormancy, half of the infected plants were kept at the Volcani Institute (central Israel) and half transported to overwinter in the Upper Galilee
- ▶ Before the next season, vines were pruned and brought back to Volcani Institute
- ▶ Plants were monitored for symptoms and infection in the next season

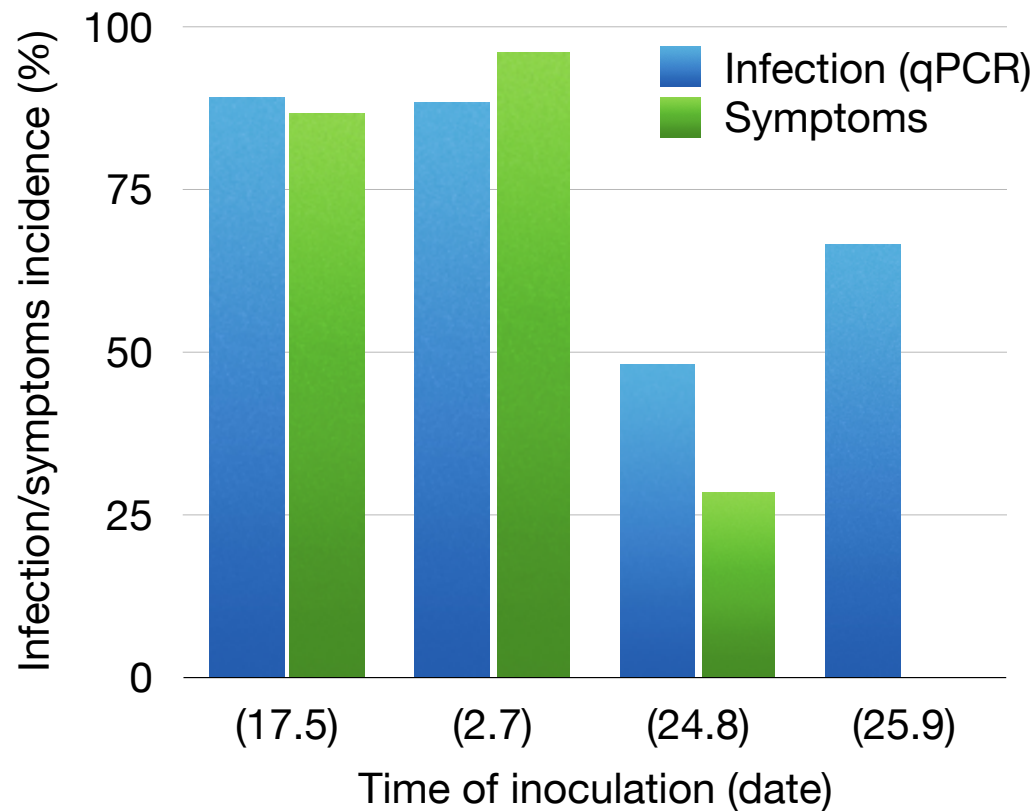


DISEASE SYMPTOMS IN THE EXPERIMENT

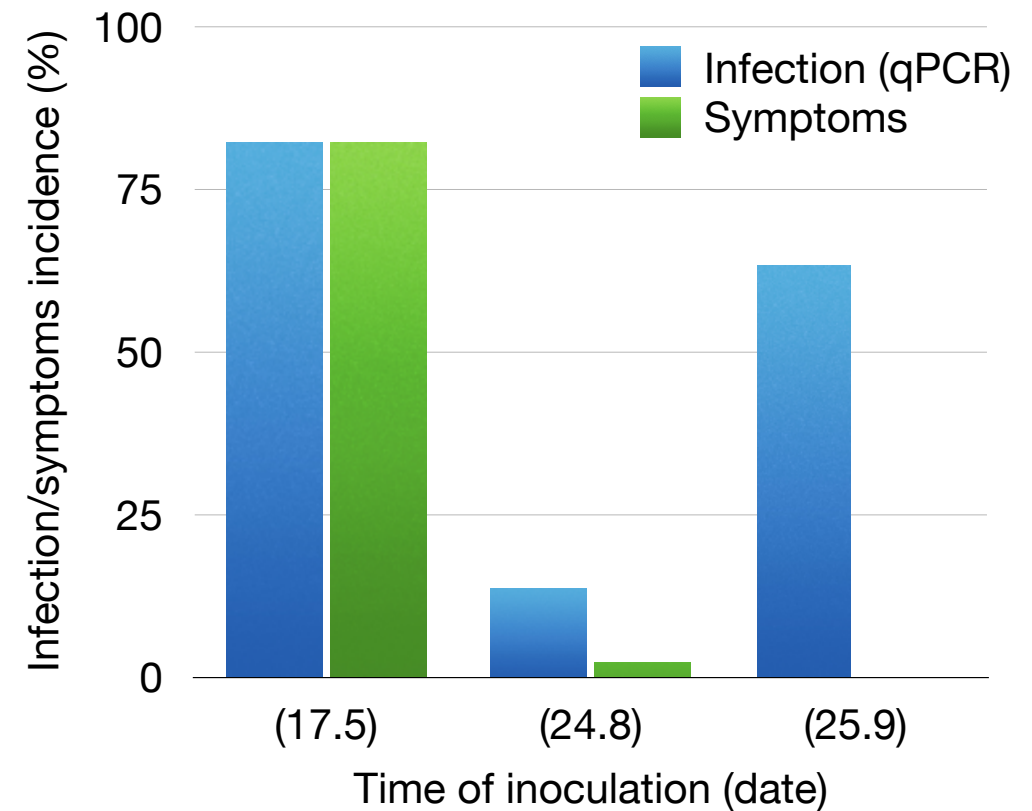


INCIDENCE OF INFECTION AND SYMPTOMATIC PLANTS

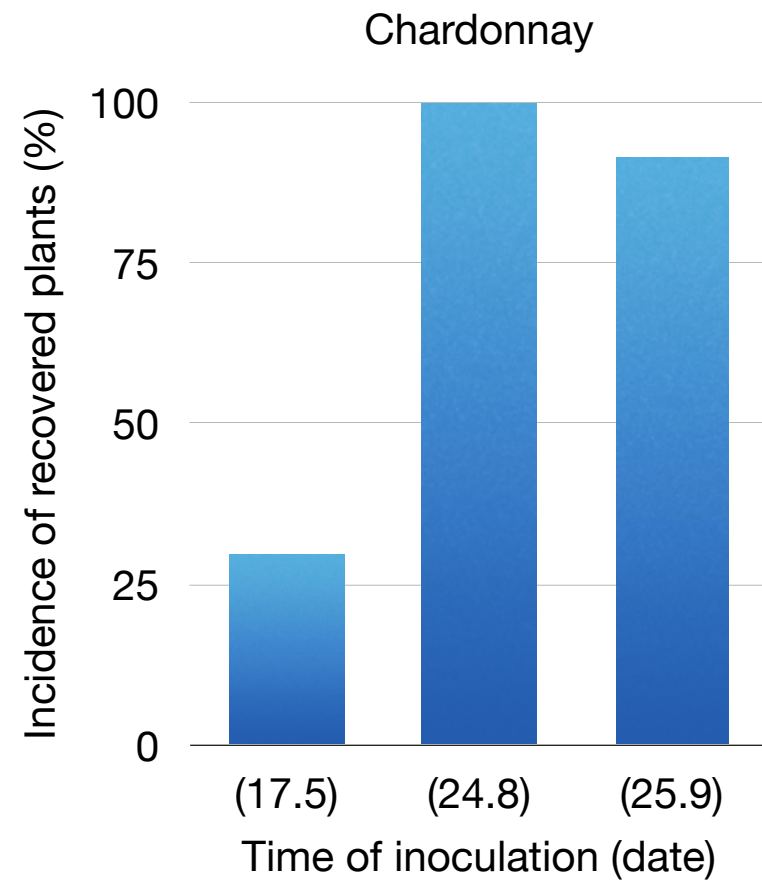
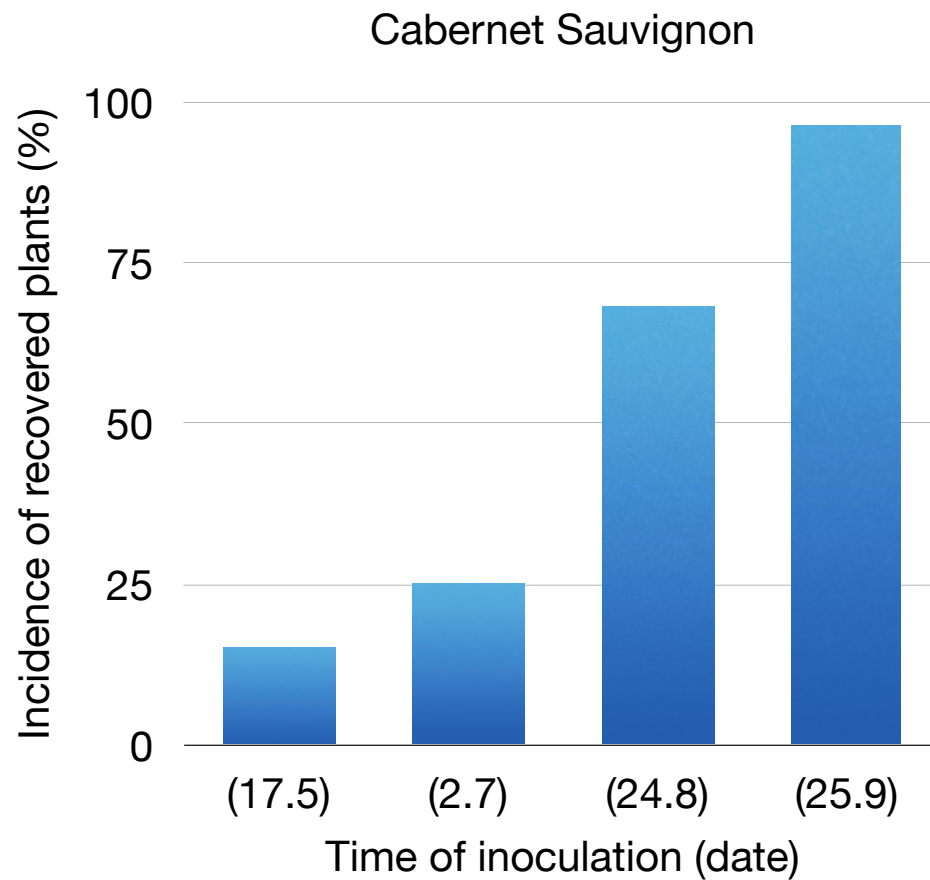
Cabernet Sauvignon



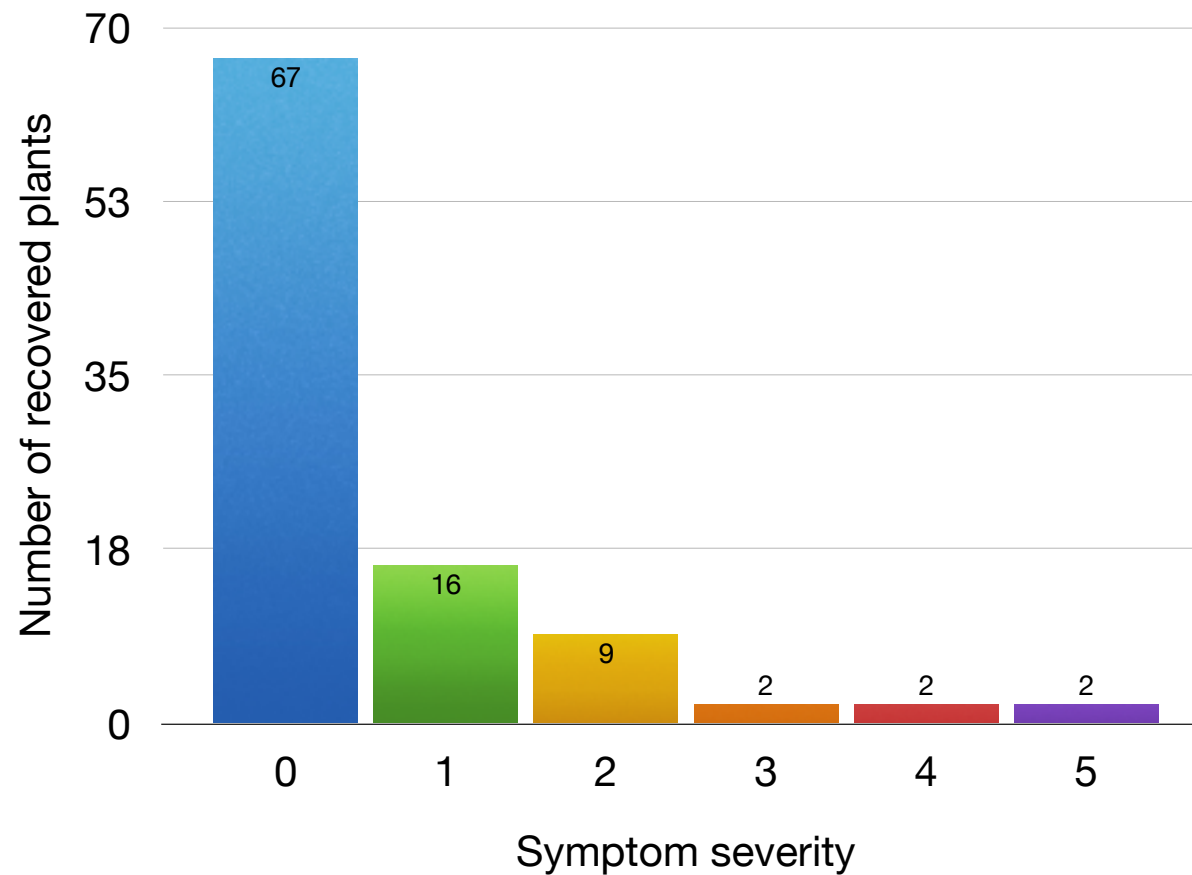
Chardonnay



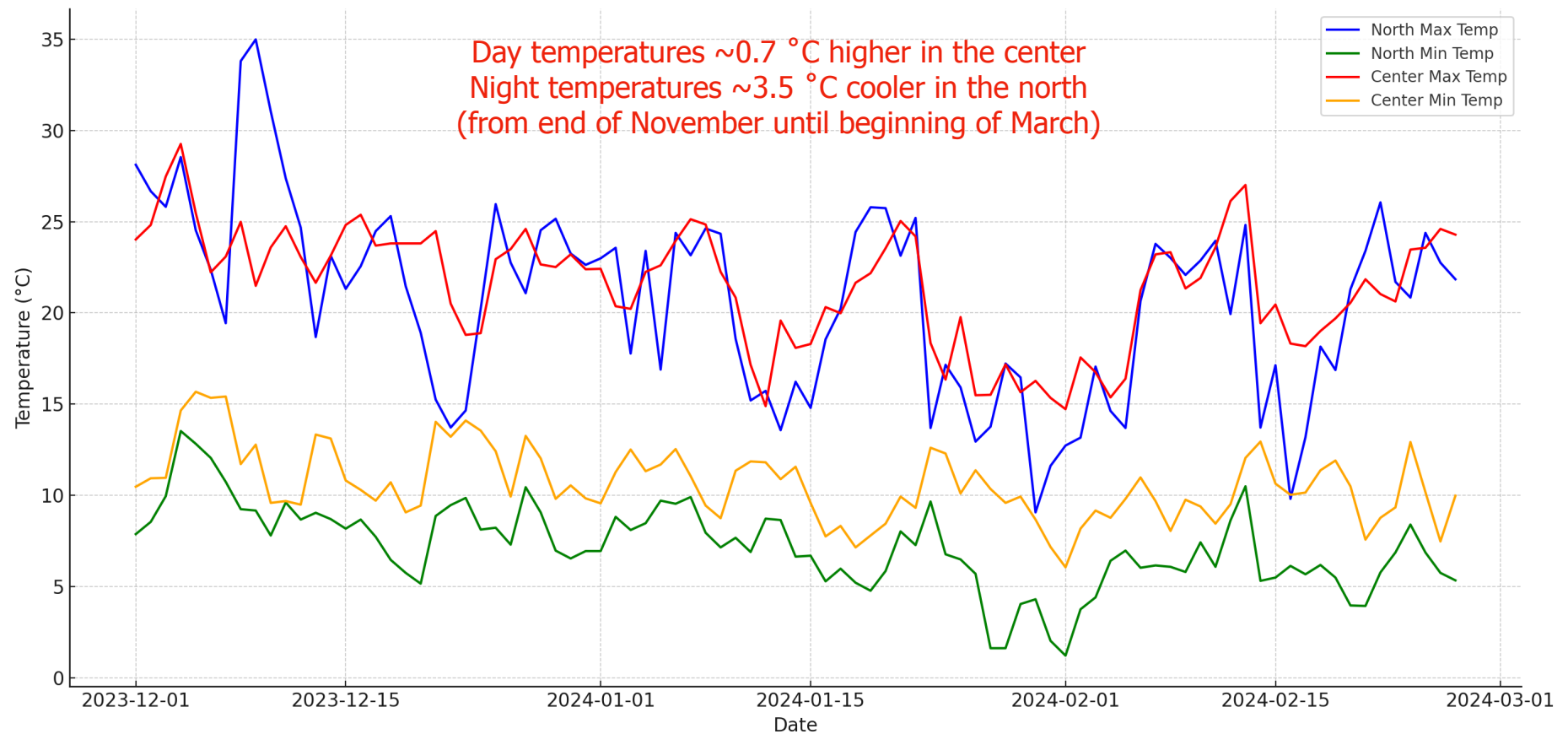
INCIDENCE OF RECOVERY IN RELATION TO TIME OF INOCULATION



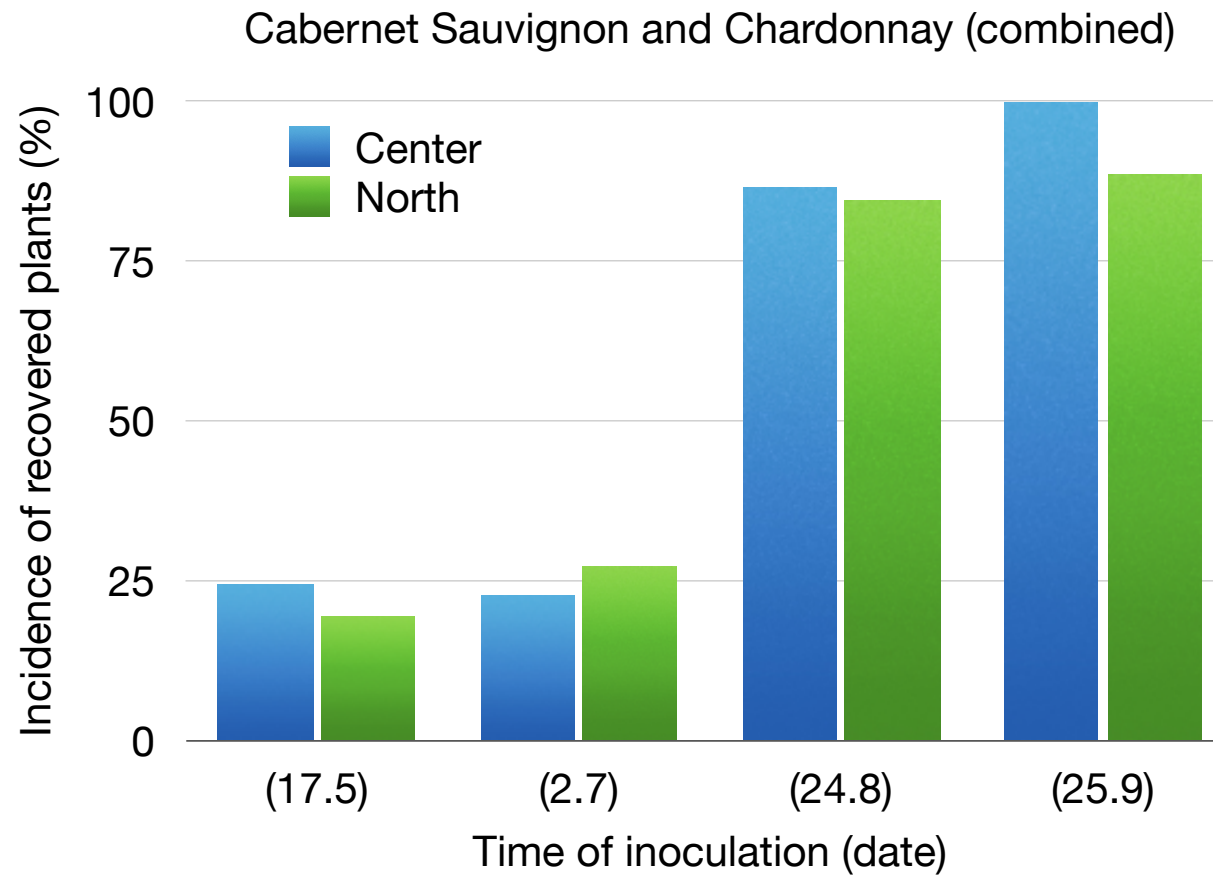
RECOVERY INCIDENCE IS CORRELATED WITH SYMPTOM SEVERITY



MAX AND MIN TEMPERATURE AT THE TWO DORMANCY SITES

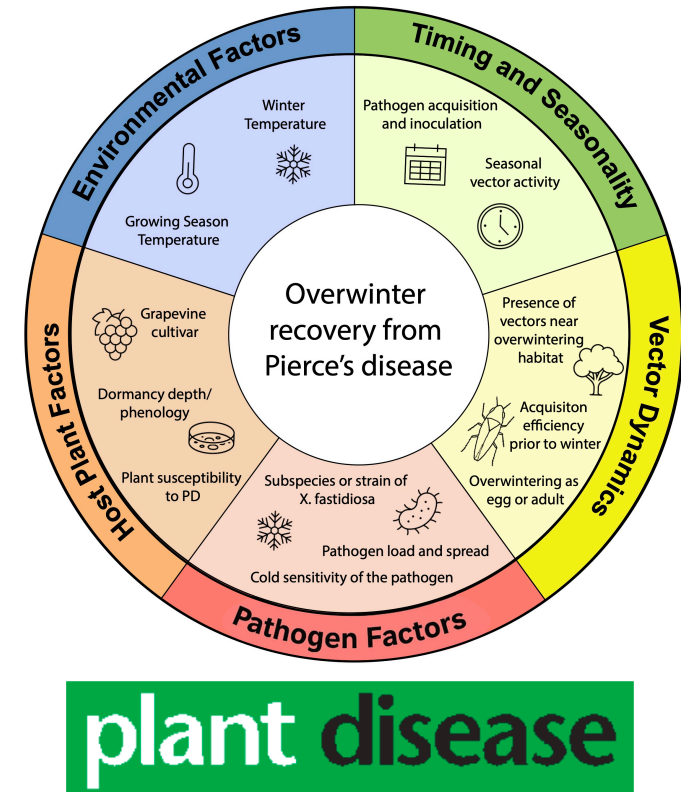


EFFECT OF SITE OF DORMANCY ON RECOVERY



CONCLUSIONS AND TAKE-HOME MESSAGE

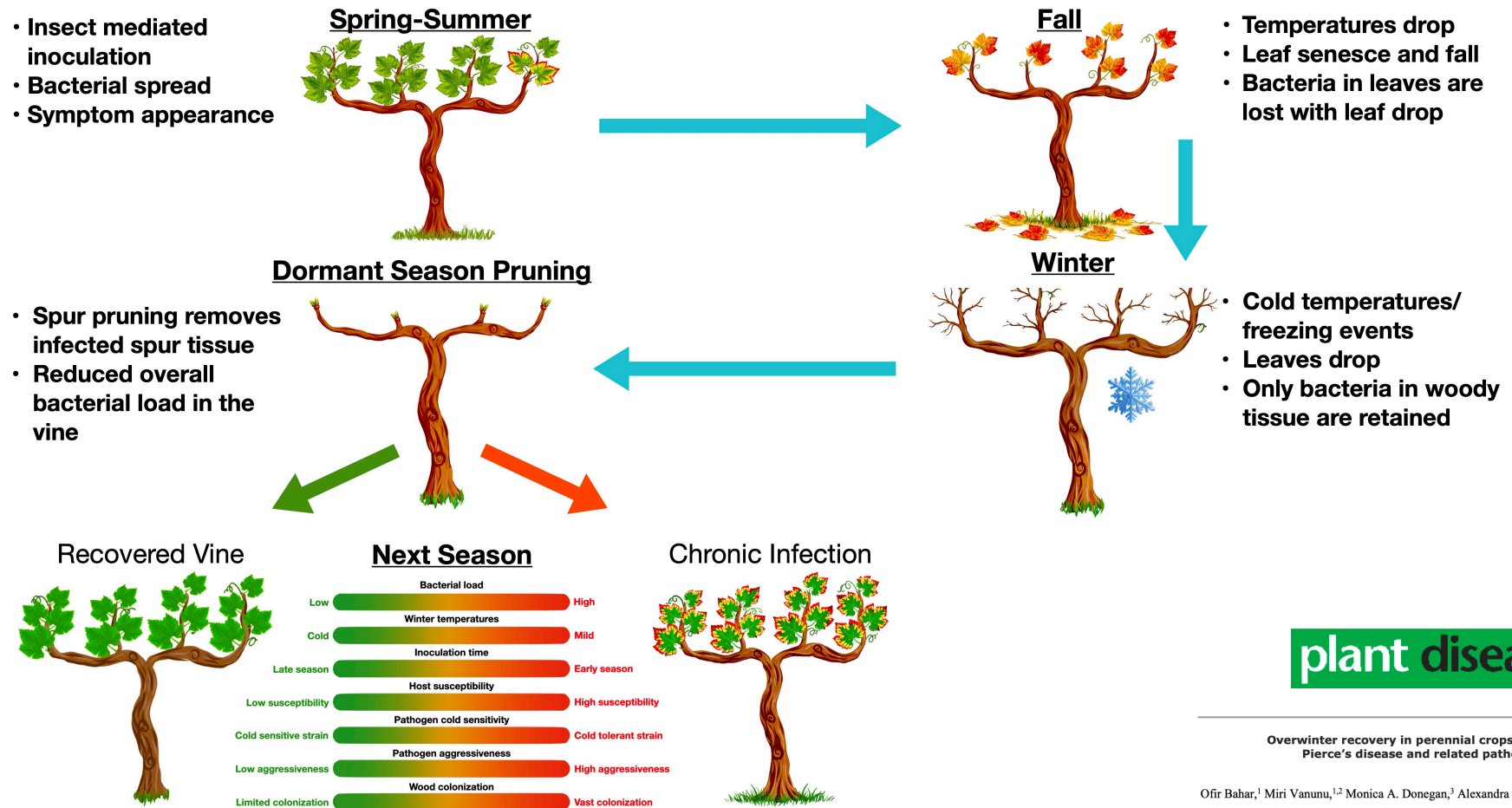
- ▶ Recovery from PD can occur under Mediterranean climate
- ▶ Early infections are more likely to become chronic
- ▶ Late infections, (August-September) have high chances to undergo overwinter recovery



**Overwinter recovery in perennial crops: insights from
Pierce's disease and related pathosystems**

Ofir Bahar,¹ Miri Vanunu,^{1,2} Monica A. Donegan,³ Alexandra Kahn,³ Andrea Brown,³ Matthew P. Daugherty,⁴ and Rodrigo P.P. Almeida³

CONCEPTUAL MODEL OF THE SEASONAL CYCLE AND FACTORS DETERMINING OVERWINTER RECOVERY FROM XF



plant disease

Overwinter recovery in perennial crops: insights from
Pierce's disease and related pathosystems

Ofir Bahar,¹ Miri Vanunu,^{1,2} Monica A. Donegan,³ Alexandra Kahn,³ Andrea Brown,³ Matthew P. Daugherty,⁴ and Rodrigo P.P. Almeida³

THANKS

Epidemiology team



Dr. Lior Blank



Prof. Dani Shtienberg



Ministry of Agriculture

Microbiology team



Noa Zecharia



Miri Vanunu



Yuval Omer



Orit Dror



Eden Amzallag



Nofar Assoline

Entomology team



Dr. Rakefet Sharon



Maor Tomer

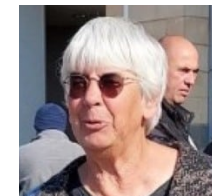


המועצה לגפן היין בישראל
Israel Wine Grapes Board

Grapevine team



Noa Maoz



Tirtza Zahavi



Plant protection and inspection services

- Ami Haberman
- Zahi David
- Nadav Ezra
- Yael Meller
- Upper Galilee grapevine growers
- Yuval Ogn and Haim Gorlik

plant disease

Overwinter recovery in perennial crops: insights from
Pierce's disease and related pathosystems

Ofir Bahar,¹ Miri Vanunu,^{1,2} Monica A. Donegan,³ Alexandra Kahn,³ Andrea Brown,³ Matthew P. Daugherty,⁴ and Rodrigo P.P. Almeida³



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

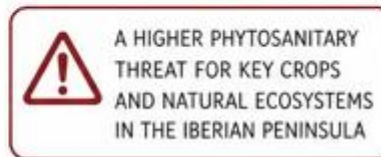
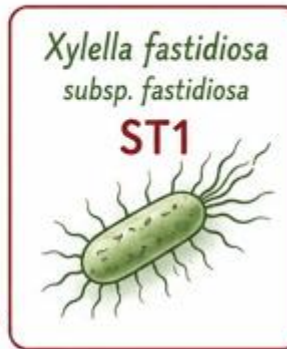
Félix Morán^{1*}; Silvia Barbé¹; Carlos Selles-Ribera¹; Adela Monterde¹; and Ester Marco-Noales¹

¹ Instituto Valenciano de Investigaciones Agrarias (IVIA), Center for Plant Protection. Bacteriology Unit, Moncada, Valencia, Spain.

* Corresponding: moran_fel@gva.es

INTRODUCTION

The recent emergence of the subspecies **fastidiosa ST1** in the Iberian Peninsula has heightened the phytosanitary threat posed by *Xf*, as ST1



The **objective** of this work is to **elucidate the origin and epidemiological context of the new outbreak detected in Extremadura (Cáceres)** by performing a molecular and phenotypic characterization of isolates from the outbreak. By sequencing and typifying these isolates using WGS and MLST, our goal is to determine their phylogenetic relationships, evaluate genetic diversity, and infer possible routes of introduction and spread in Spain.

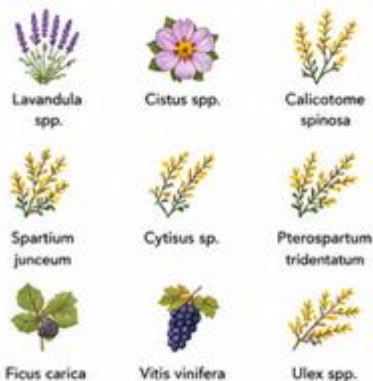
METODOLOGY

1

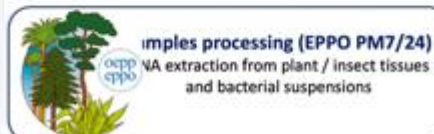
Plant and Insect Material

Samples collected in Extremadura in 2024-2025 in high-risk areas near to Portugal

Plant Material



Insect Material Potential vectors



2

Isolation of *Xylella fastidiosa*

Extracts plated on BCYE medium (PM7/24)



Incubation at 28 °C
12-20 days- 6 weeks

Colonies with morphology compatible with *X. fastidiosa*



Purification on BCYE

3

Isolate-based assays (from purified isolates)

Phage Typing Assay (PTA)



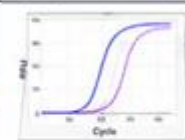
Spot Test (Selles-Ribera et al., 2026)



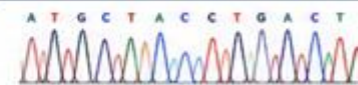
Incubation at 28 °C
up to 7 days

Detection by qPCR
(Harper et al., 2007)

Subspecies identification
(Dupas et al., 2019)



MLST
(Scaelly et al., 2005 and Yuan et al., 2010)



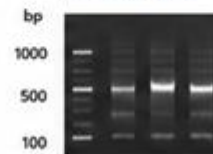
Sequence Type (ST) assignment
(PubMLST database)

4

Plasmid detection and chromosomal integration

Plasmid detection (PCR)

Primers pairs targeting plasmid associated with subsp. *fastidiosa* (Velasco-Amo et al., 2022 and Van Horn et al., 2019)



pXFLS21 chromosomal integration (PCR)

Junctions and primer positions



Confirms
chromosomal-plasmid junctions

5

WGS and comparative genomics

Hybrid Sequencing Approach

Oxford Nanopore (Long reads)
Illumina (Short reads)



Hybrid assembly, QC and Annotation
(Hybracter, Fastp, Fastplong and Prokka)

Plasmid Identification
PlasmidFinder

ANI and Phylogeny
CLC Genomics Workbench v25.0

Pangenome and Core-genome
(Roary, Panaroo and Gubbins)

Prophage prediction
PHAGE Search Tool (PHAST)

6

Pathogenicity assay

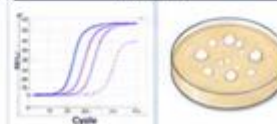
Inoculation of strain IVIA 906/24



Vitis vinifera cv. Tempranillo
-S Xff-inoculated
-S Controls
-BSL-2 greenhouse conditions
-Monitoring weekly

Sampling at 30, 92 and 122 dpi
and symptomatic stages

Detection by qPCR
(Harper et al., 2007) and Bacterial isolation



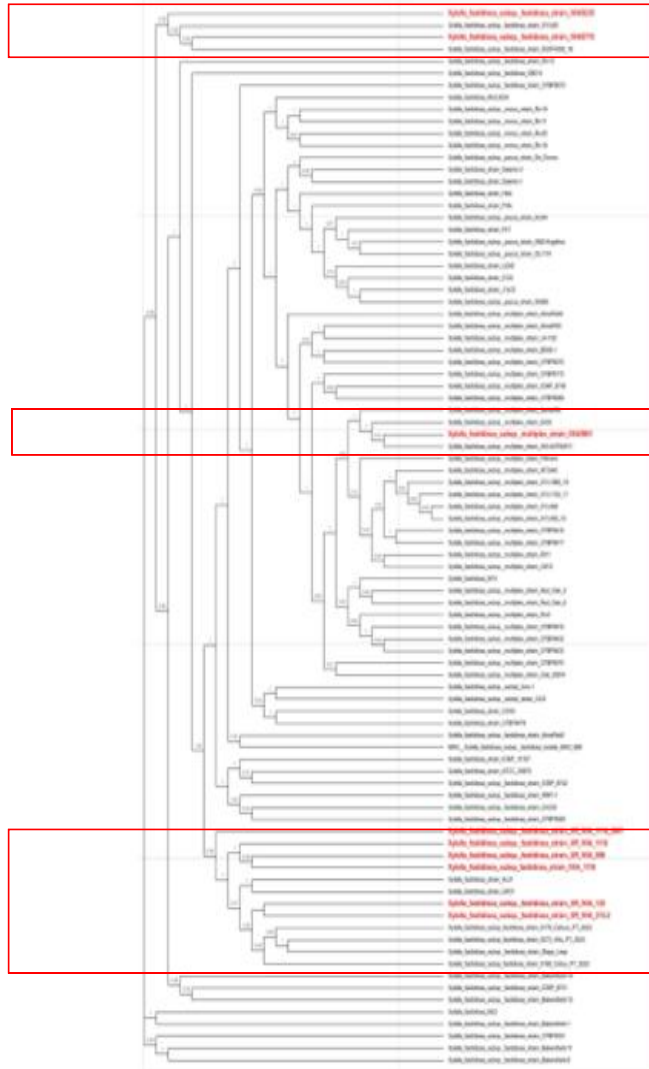
RESULTS

	IVIA-906/24	IVIA-1116/24	IVIA-1118/24	IVIA-1119/24	IVIA-125/25	IVIA-313.2/25
Sequencing statistics¹						
Oxford Nanopore Technology reads	592,862	373,219	373,494	269,617	306,593	809,763
Illumina paired reads	3,583,588	6,090,778	8,231,502	7,674,339	-	-
Assembly statistics						
Hybrid assembly	Yes	Yes	Yes	Yes	No	No
Nº <i>De novo</i> contigs	1	1	1	10	15	1
Genome Size	2,534,294 bp	2,532,475 bp	2,533,848 bp	2,536,606 bp	2,554,241 bp	2,551,738 bp
GC content (%)	51.7% (1,309,677 nt)	51.7% (1,308,651 nt)	51.7% (1,309,429 nt)	51.5 % (1,311,661 nt)	51.9 % (1,324,931 nt)	51.8% (1,320,588 nt)
Genome completeness²	99.64%	99.64%	99.64%	98%	99.64%	99.75%
Genome coverage	1161X	1188X	1218X	1288X	118X	909X
Presence plasmid	Negative	Negative	Negative	Negative	Negative	Negative
Annotation (Prokka v1.14.6)						
CDS³	2,419	2,422	2,422	2,401	2,440	2,453
rRNA⁴	6	6	6	6	9	6
tmRNA⁵	1	1	1	1	1	1
tRNA⁶	46	49	49	49	51	49
GenBank Number	NZ_CM155230.1	NZ_CM155228.1	NZ_CM155229.1	-	-	-

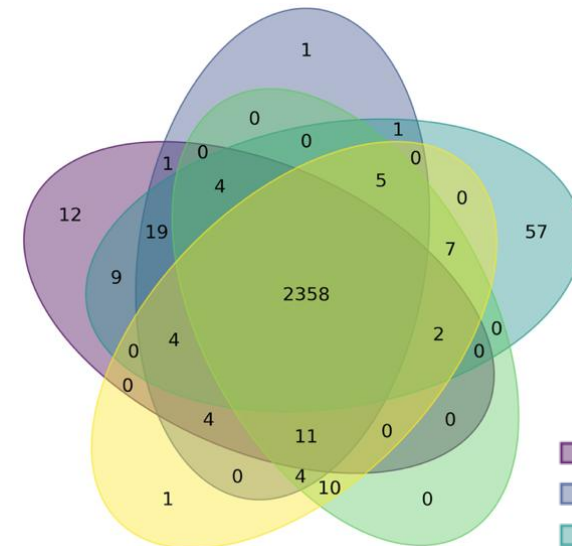
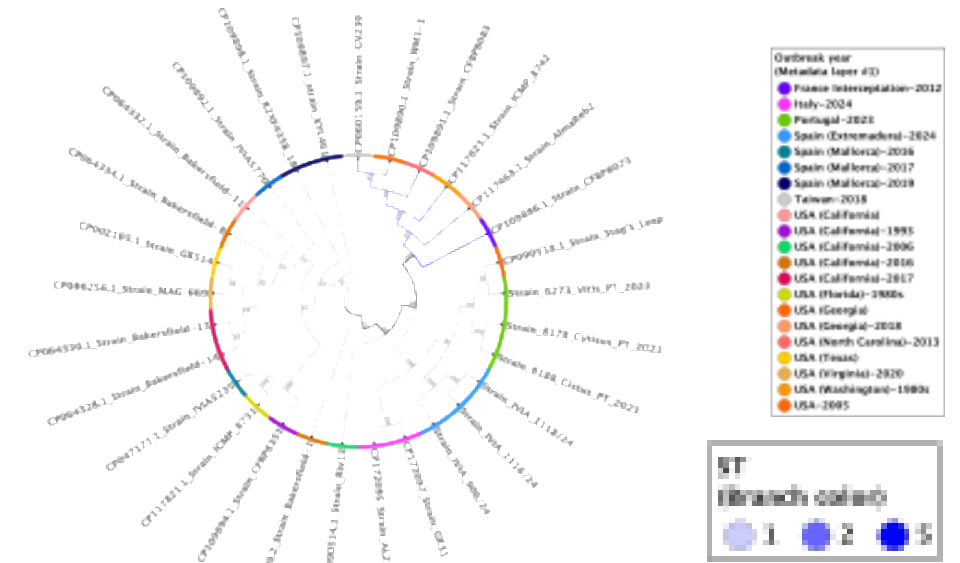
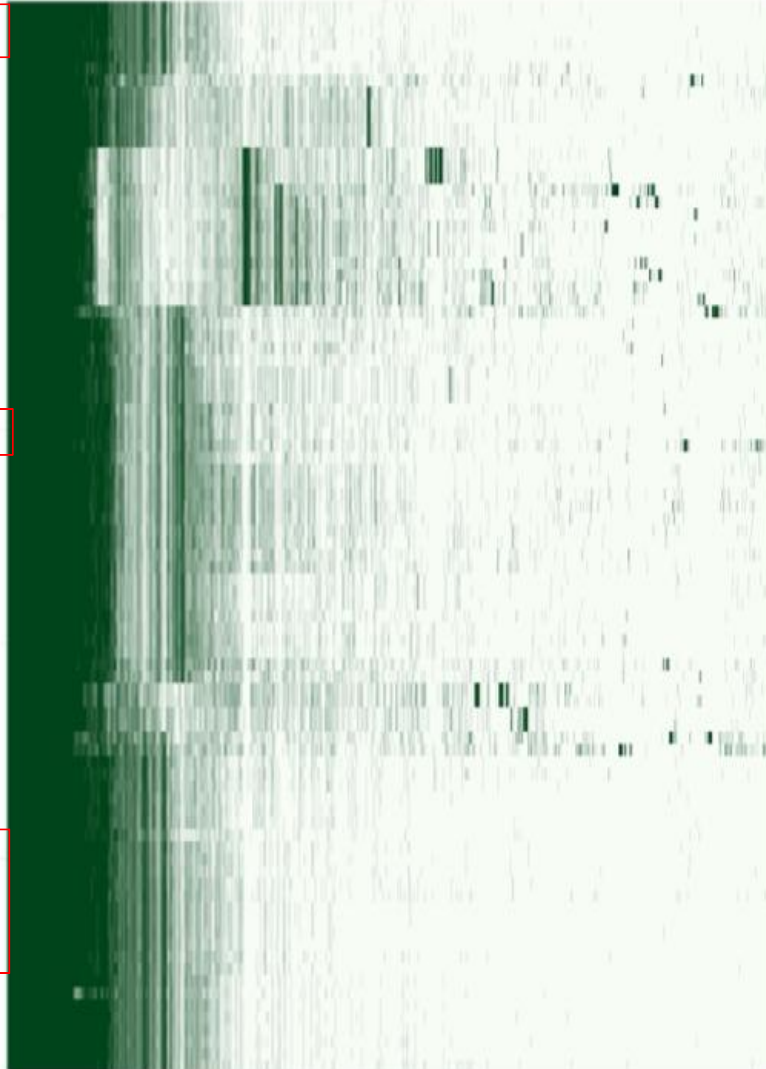
RESULTS

Core-genome analysis

Gene content dendrogram
of 88 *Xylella fastidiosa* strains



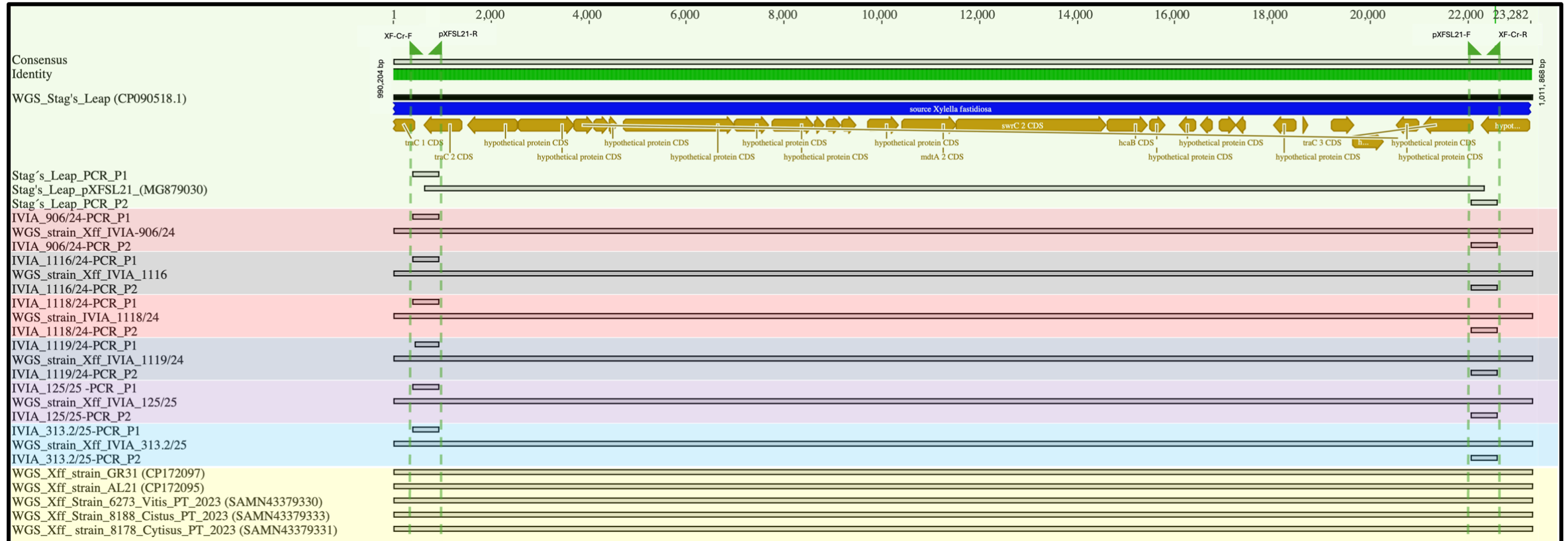
Roary matrix (11327 gene clusters)



- Stag's Leap
- 6273_Vitis_PT_2023
- BA_GR31
- IVIA-906/24
- IVIA-1118/24

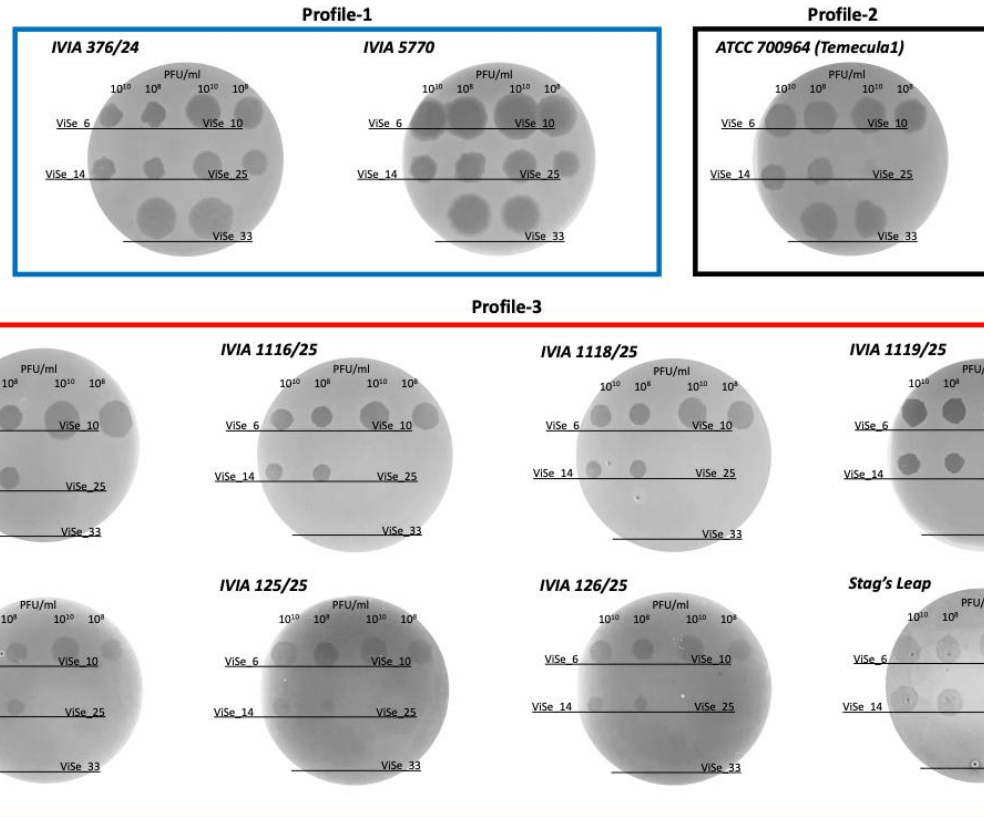
RESULTS

Chromosomal integration of plasmid pXFSL21

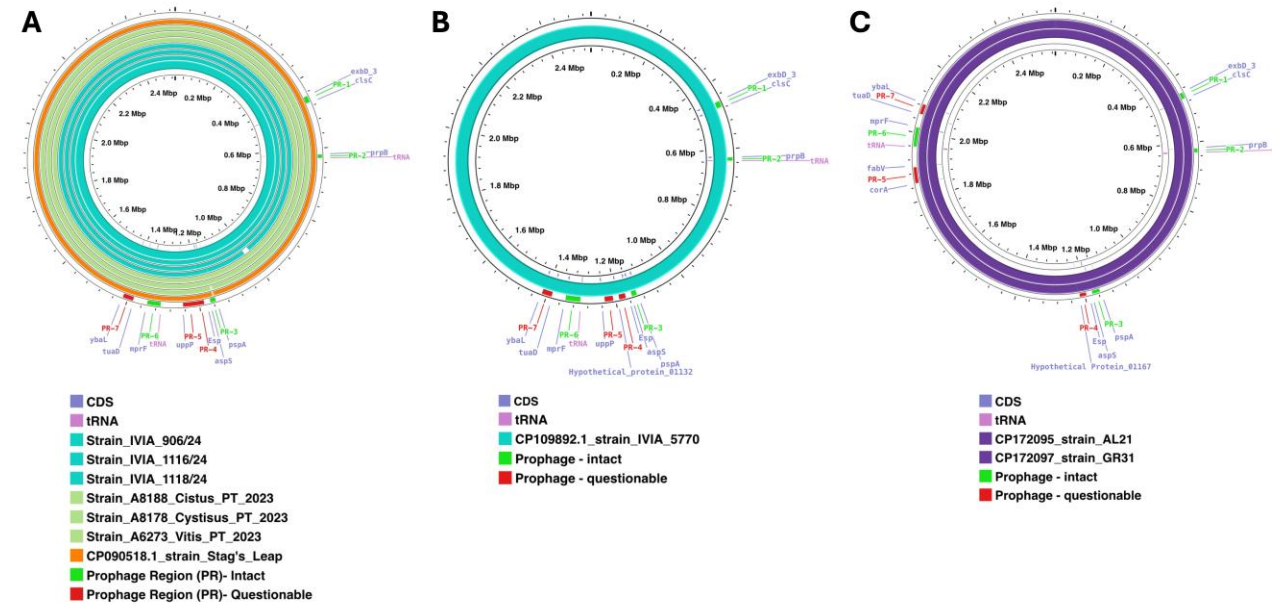


RESULTS

Phage Typing

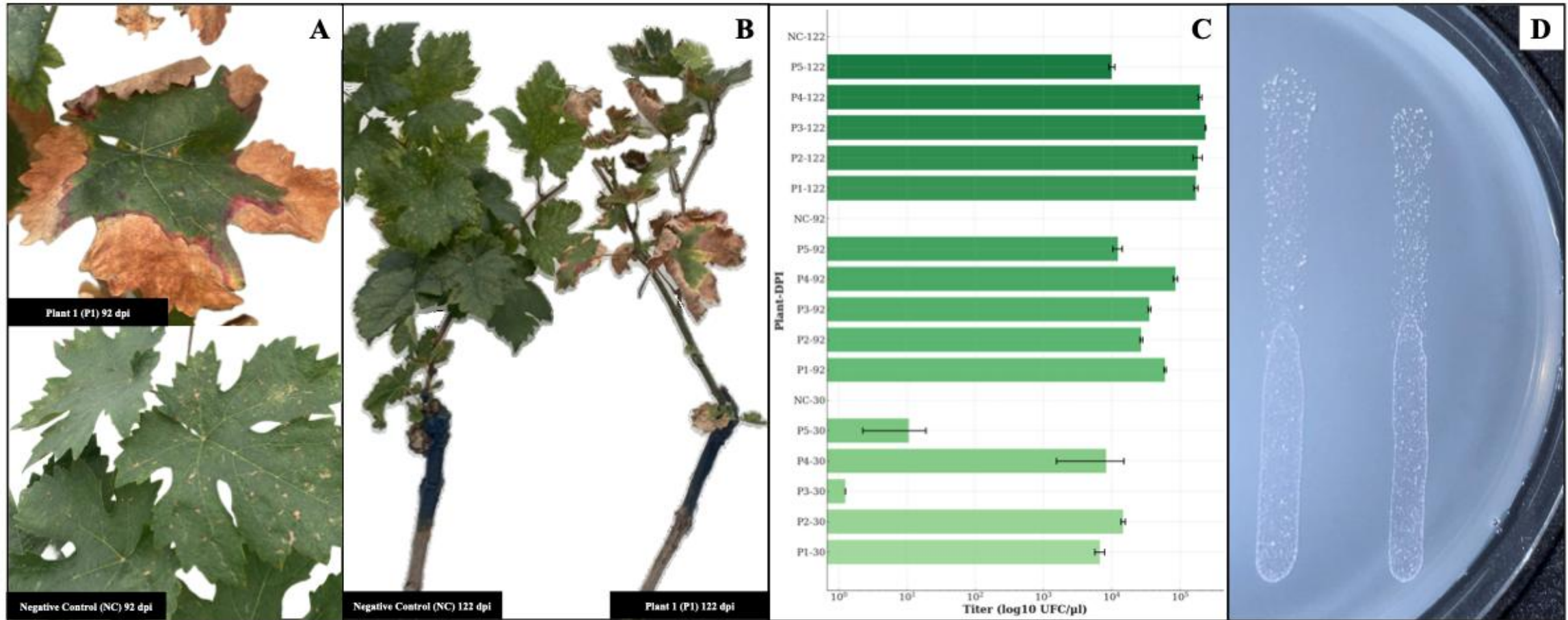


Prophages analysis



RESULTS

Pathogenicity assay





CONCLUSIONS

- **Close relationship between recent European outbreaks and American** strains, confirming the ongoing vulnerability of European agriculture to the introduction of new variants.
- **The full chromosomal integration of plasmid pXFSL21** in IVIA isolates, together with its conservation in Italian and North American ST1 strains, supports its value as a stable genomic marker for tracing the dissemination and common origin of the ST1 lineage.
- **Phage-based typing revealed a consistent lytic profile** among Extremadura isolates, distinct from Mallorca ST1 strains and the reference strain Temecula1, indicating phenotypic variability within this lineage.
- **Systematic surveillance through WGS should be consolidated as a central tool** for detection, traceability, and supporting the design of effective containment strategies against future epidemic episodes of *Xylella fastidiosa* outbreaks.

Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026

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

Authors: Giorgia Tranchina⁺⁺¹, Aparna S. Balan⁺⁺¹, Serafina Serena Amoia², Angelo Sicilia¹, Floriana Bonanno¹, Maria Saponari², Annalisa Giampetruzzi², Irene Granata¹, Francesco Paolo Marra¹, Tiziano Caruso¹, Annalisa Marchese¹

Affiliations

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⁺Both authors contributed equally

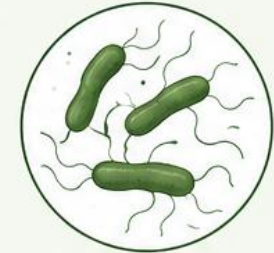
^{*}Corresponding authors: giorgia.tranchina@unipa.it





OBJECTIVE

To evaluate the gene expression response of **three almond (*Prunus dulcis*) varieties** **48 hours** after inoculation with different strains of *Xylella fastidiosa*, and to integrate **transcriptomic** data with **SNP analysis** to identify **molecular markers** associated with early response.



Xylella fastidiosa

PLANT MATERIAL

Three almond (*Prunus dulcis*) varieties

Avijor



Pizzuta



Tuono



×

INOCULATION

Xylella fastidiosa strains

ST1



ST26

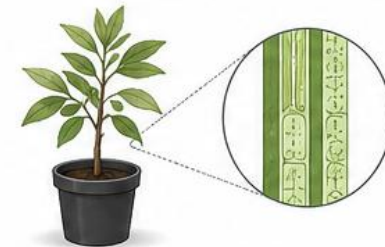


ST53

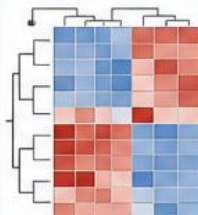


SAMPLING

48 hours post-inoculation

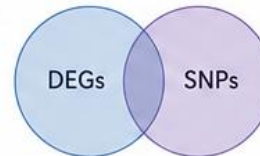


TRANSCRIPTOMIC ANALYSIS (RNA-Seq)

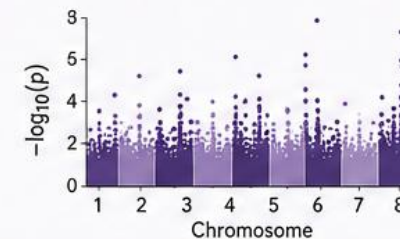


Identification of Differentially Expressed Genes (DEGs)

DATA INTEGRATION



SNP ANALYSIS (Axiom 60K array)



Identification of SNPs associated with early response



CORRELATION OF DEGs AND SNPs



Identification of molecular markers associated with the early response to *Xylella fastidiosa* in almond

RESULTS

Preliminary Transcriptomic Analysis

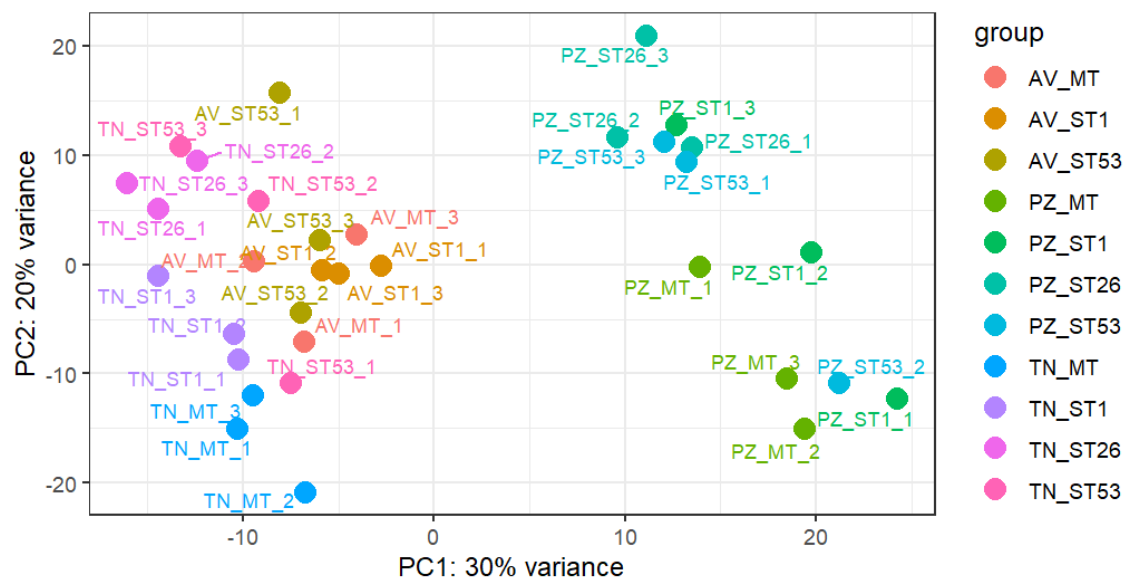


Figure 1. Principal Component Analysis (PCA) of transcriptomic profiles in almond cultivars. Samples cluster primarily by genotype along PC1 (30% variance), with Pizzuta d'Avola (PZ) separating from Tuono (TN) and Avijor (AV). MT indicates control samples; ST groups correspond to different *Xylella fastidiosa* strains.

GENERAL OVERVIEW OF DEGs

Differentially expressed genes across multiple comparisons

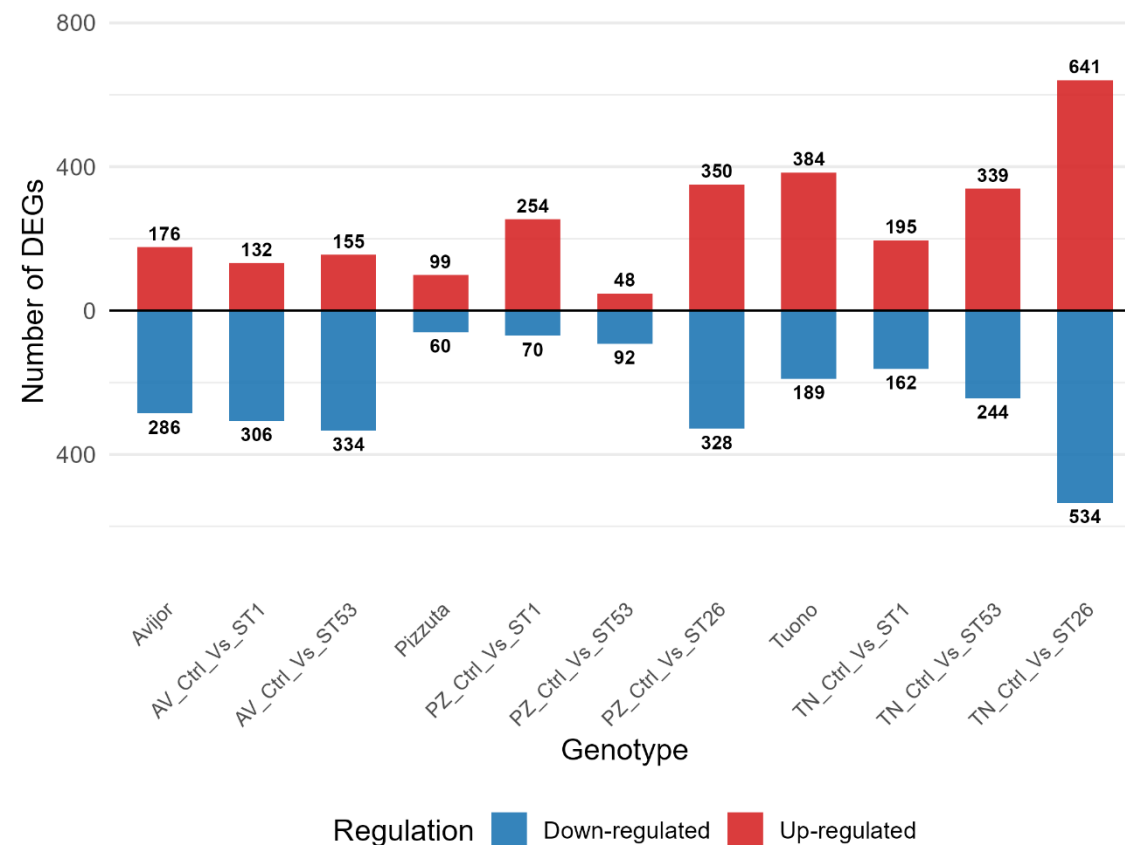
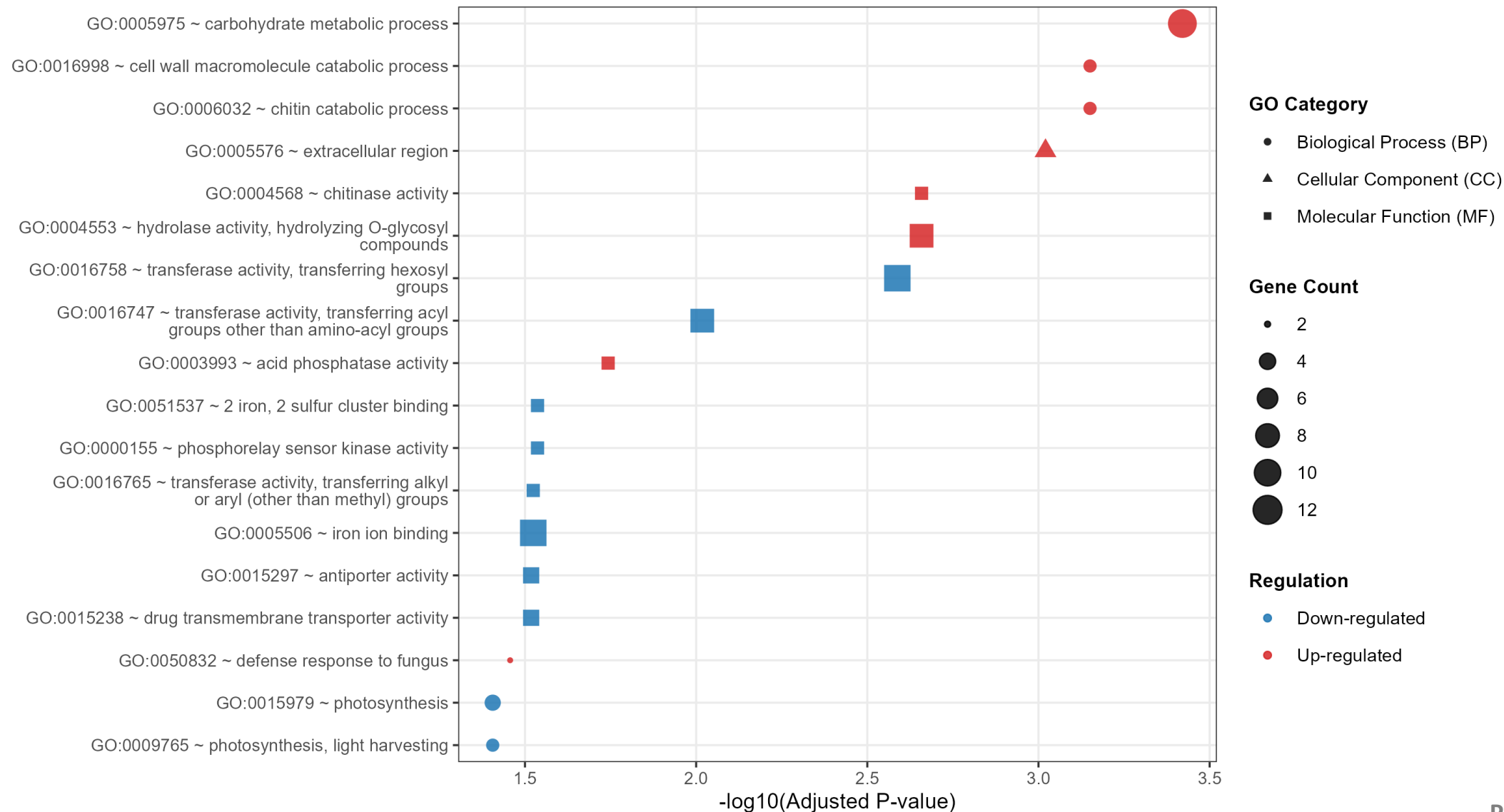


Figure 2. The mirror barplot quantifies up-regulated (red) and down-regulated (blue) genes at 48 hours post-inoculation.

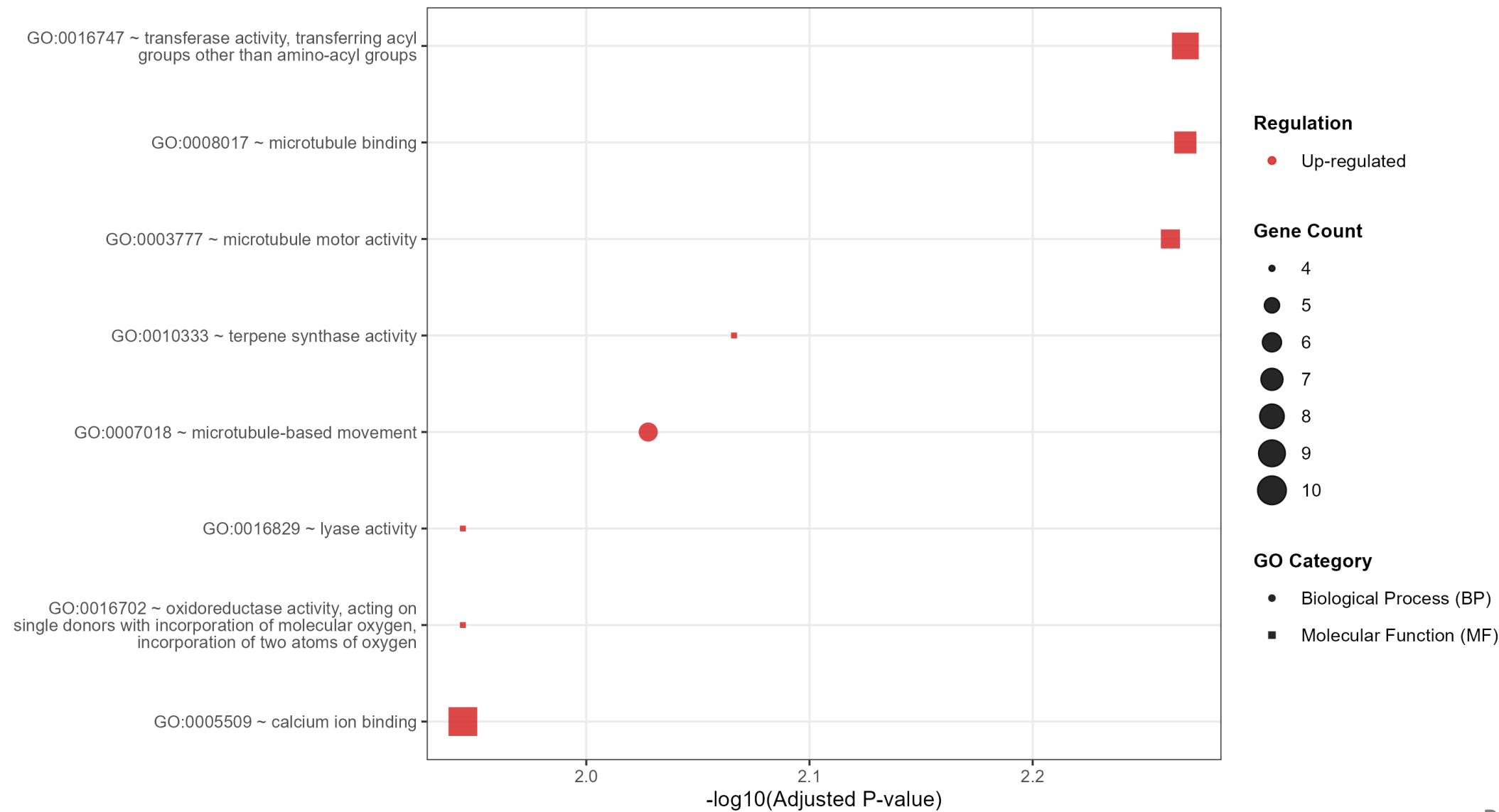
RESULTS

GO Enrichment Analysis - Avijor



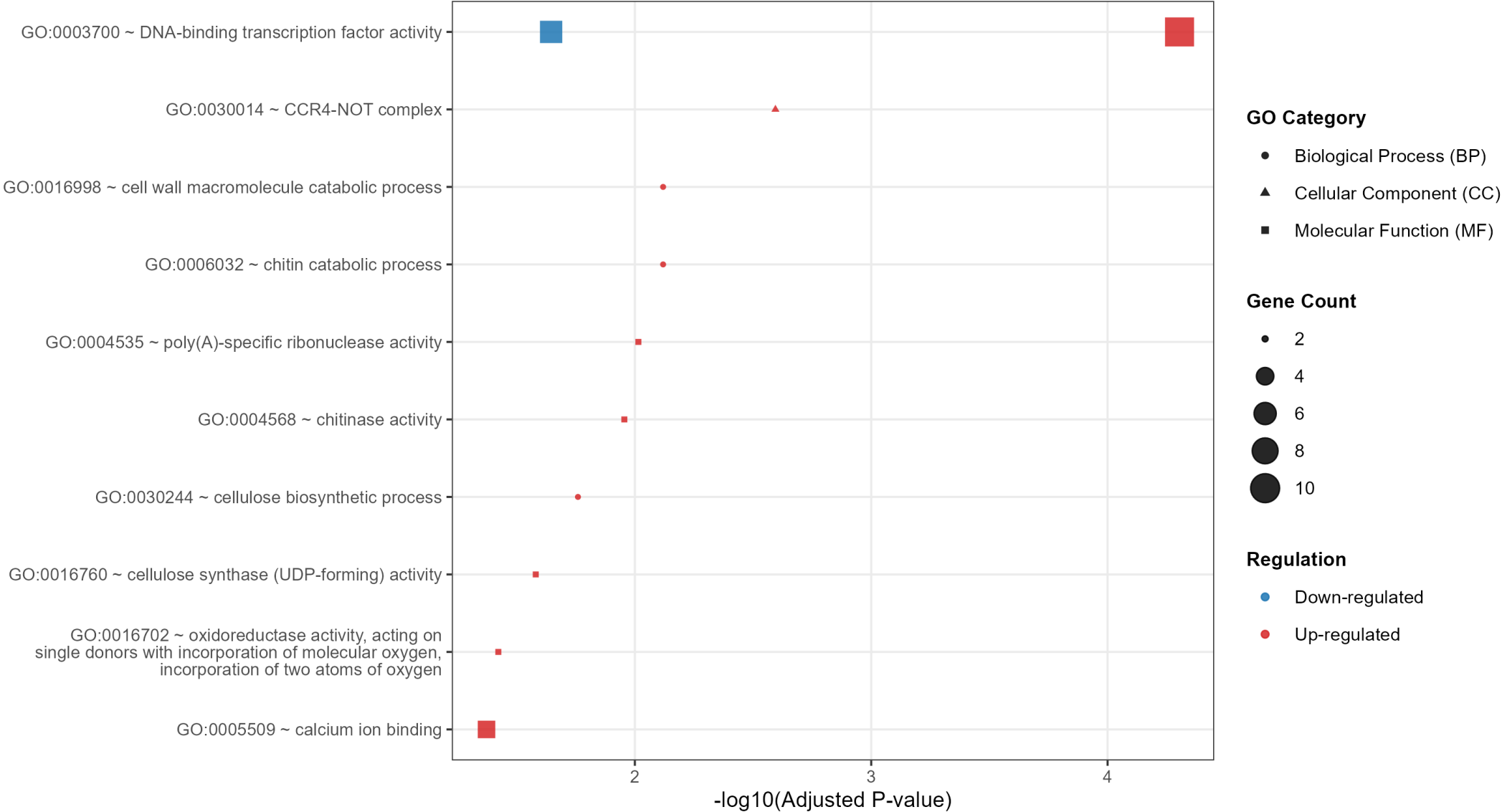
RESULTS

GO Enrichment Analysis - Tuono



RESULTS

GO Enrichment Analysis - Pizzuta



RESULTS

SNP Distribution within DEGs across almond cultivars and *Xylella fastidiosa* strains

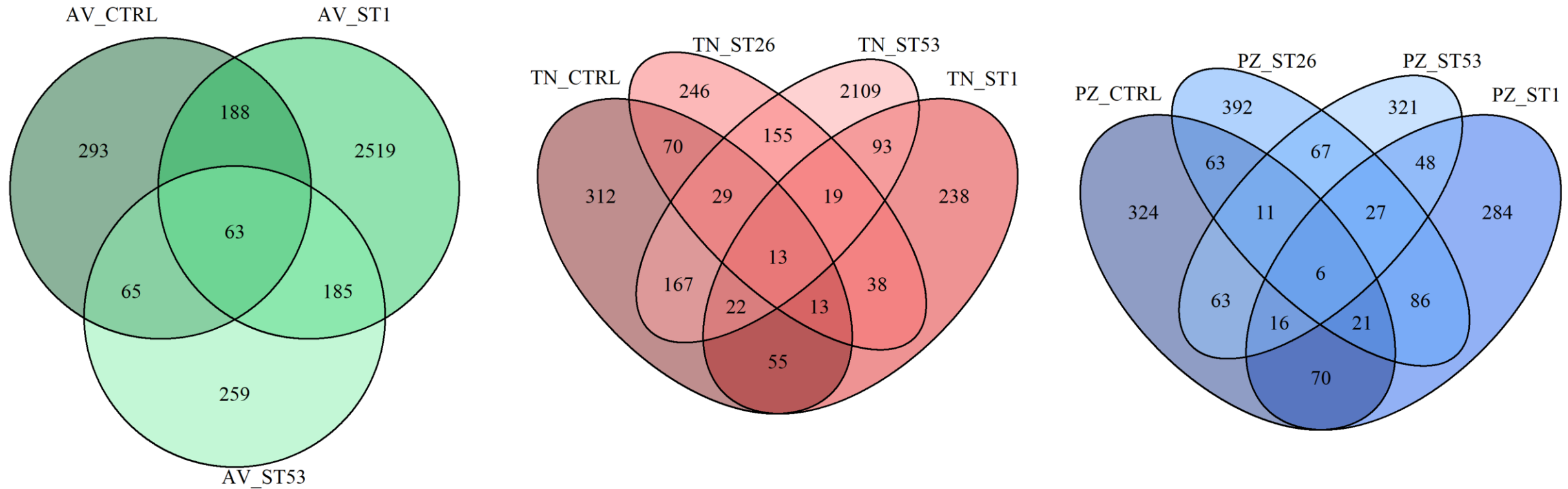
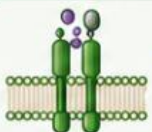


Figure 3. Venn diagrams showing shared and unique SNPs identified within differentially expressed genes among control and inoculated samples with different *Xylella fastidiosa* strains.

WORKING MODEL OF ALMOND RESPONSES TO *Xylella fastidiosa* INFECTION

PIZZUTA D'AVOLA AND AVIJOR

COORDINATED DEFENSE-ASSOCIATED RESPONSE



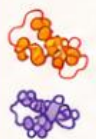
1. PATHOGEN PERCEPTION AND RECOGNITION

Recognition of *Xylella fastidiosa* by pattern recognition receptors (NLRs, RLKs, LRR-RLKs)
→ Early detection of the pathogen



2. DEFENSE SIGNALING ACTIVATION

Activation of defense-related transcription factors (WRKY, MYB, NAC, AP2/ERF and others)
→ Induction of downstream defense pathways



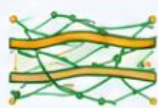
3. PR-PROTEIN ACTIVATION

Induction of pathogenesis-related proteins (e.g., chitinases, β -1,3-glucanases, PR proteins)
→ Direct antimicrobial activities



4. REDOX HOMEOSTASIS

Activation of antioxidant and redox-related enzymes (peroxidases, glutaredoxins, thioredoxins, oxidoreductases)
→ ROS balance and signaling



5. CELL WALL REMODELING AND FORTIFICATION

Up-regulation of cell wall-associated genes (pectin-related proteins, cellulases, GDSL lipases, peroxidases, xyloglucan endotransglucosylases)
→ Reinforcement of physical barriers



6. STRUCTURAL REINFORCEMENT AND CONTAINMENT

Strengthening of xylem structure and limitation of bacterial spread
→ Localized defense and containment



7. PUTATIVE ENHANCED TOLERANCE TO *Xylella fastidiosa*

More coordinated and multi-layered defense response
(Pizzuta d'Avola and Avijor)



The transcriptional responses observed suggest distinct strategies adopted by the cultivars following *Xylella fastidiosa* infection. Pizzuta d'Avola and Avijor exhibit a more coordinated defense-associated transcriptional program, whereas Tuono shows a broader stress-associated response. The biological relevance of these differences and their relationship with disease outcome remain to be experimentally validated.



TUONO

BROAD STRESS-ASSOCIATED TRANSCRIPTIONAL RESPONSE



1. *Xylella fastidiosa* INFECTION

Perception of the pathogen and disruption of cellular homeostasis
→ Triggering of general stress responses



2. BROAD STRESS-ASSOCIATED TRANSCRIPTIONAL RESPONSE

- Activation of redox- and ROS-related pathways
- Extensive activation of transcription factors (WRKY, NAC, MYB, AP2/ERF and others)
- Metabolic reprogramming and general stress adaptation
- Regulation of transport-related genes and cellular homeostasis



3. LESS EVIDENT COORDINATED DEFENSE ACTIVATION

- Fewer transcripts related to PR proteins are detected
- Less apparent activation of structural defense pathways (cell wall remodeling, fortification)
- Defense response appears less focused on pathogen containment



4. WORKING HYPOTHESIS

The response of Tuono appears to rely predominantly on general stress-response pathways, with a less evident activation of some defense-related modules. Additional analyses are required to determine how this transcriptional strategy influences bacterial colonization and disease outcome.



5. PUTATIVE DIFFERENTIAL RESPONSE TO *Xylella fastidiosa*

The biological significance of the observed transcriptional profile remains to be experimentally validated through bacterial quantification, symptom assessment, and additional infection stages.

Acknowledgements



**Università
degli Studi
di Palermo**

IPSP-CNR Bari





Xylella fastidiosa

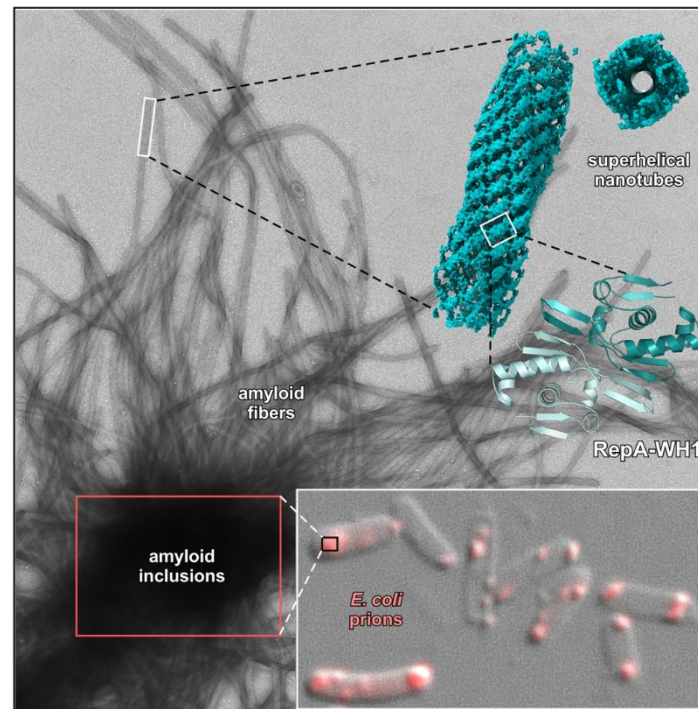
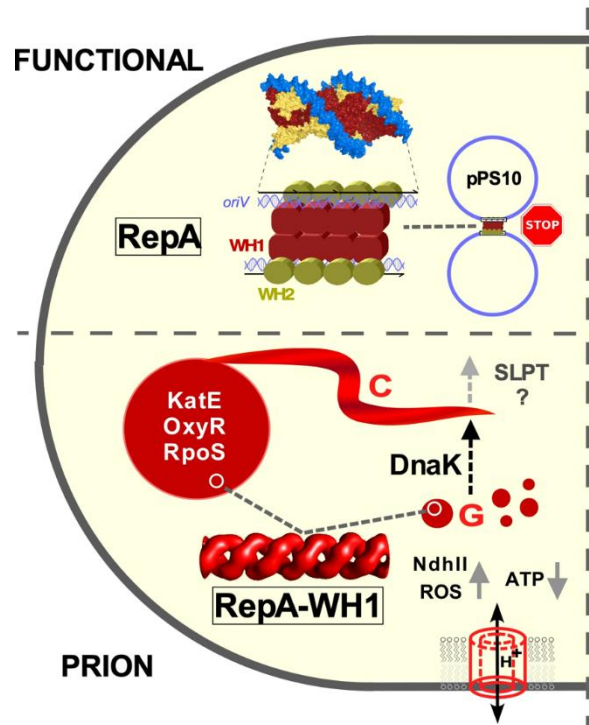
5th European conference

Bari, Italy | 22-26 June 2026



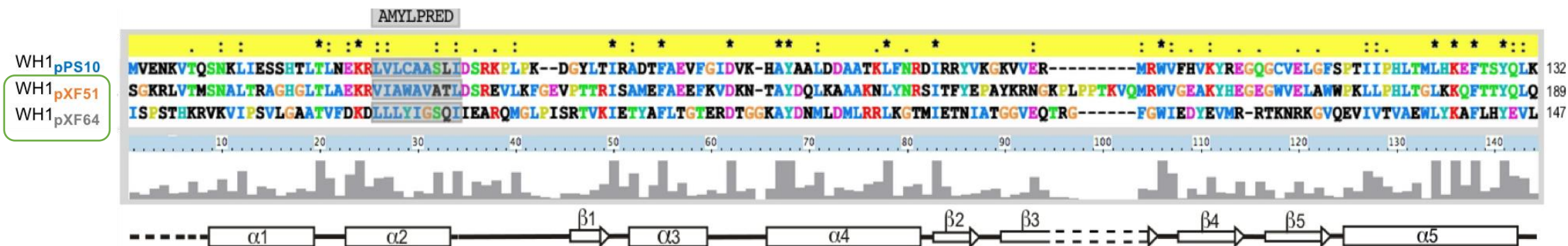
REGIONE PUGLIA

The toxicity of the WH1 domains from replication proteins on bacteria



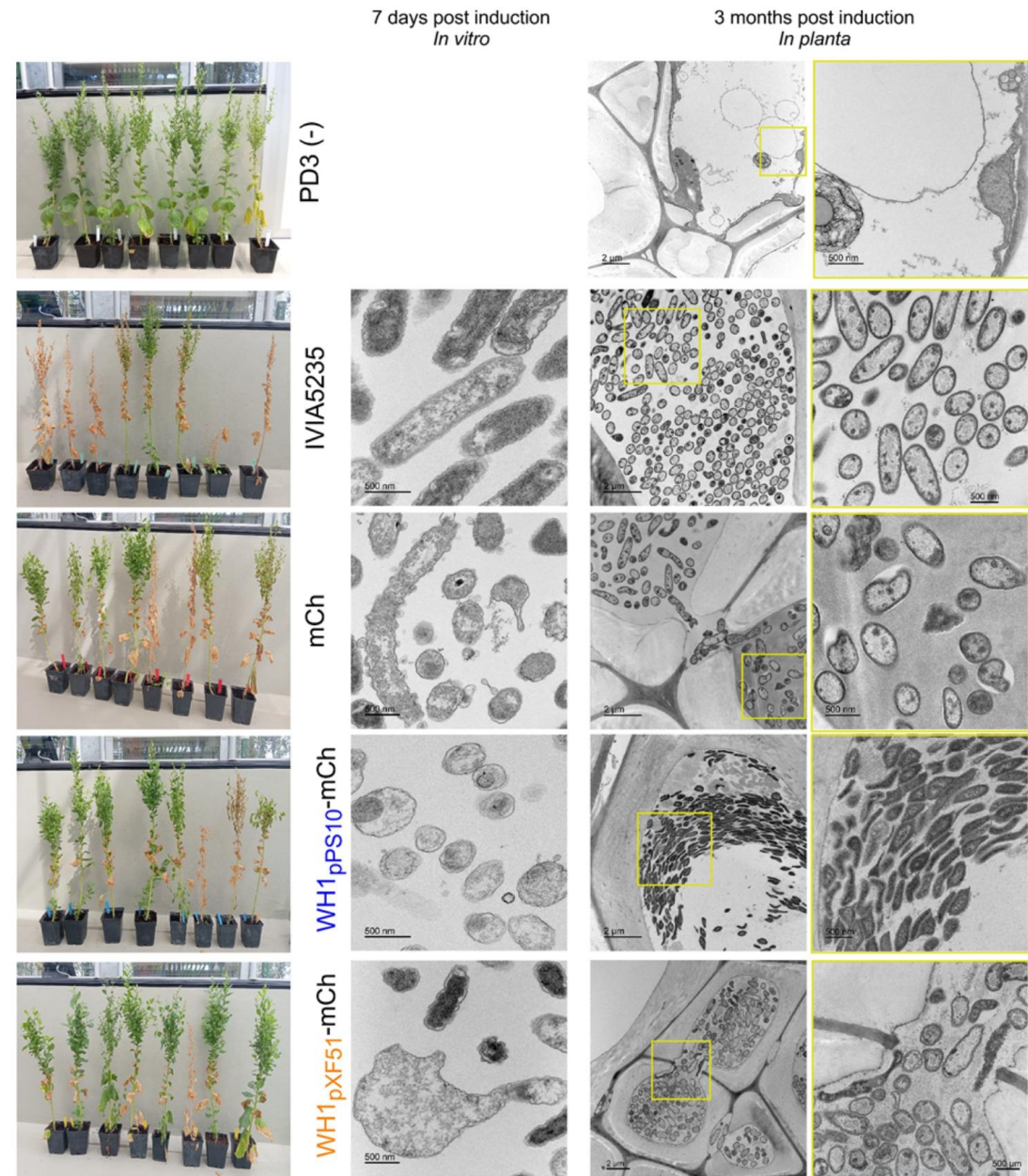
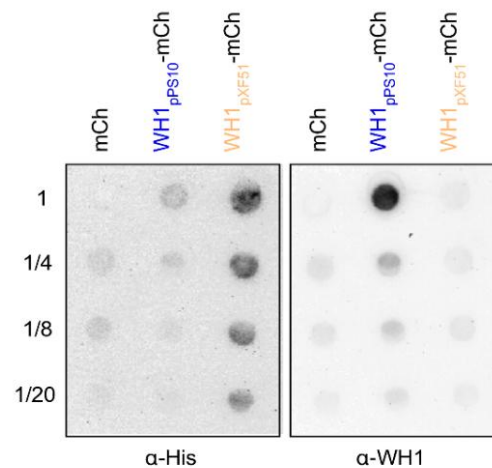
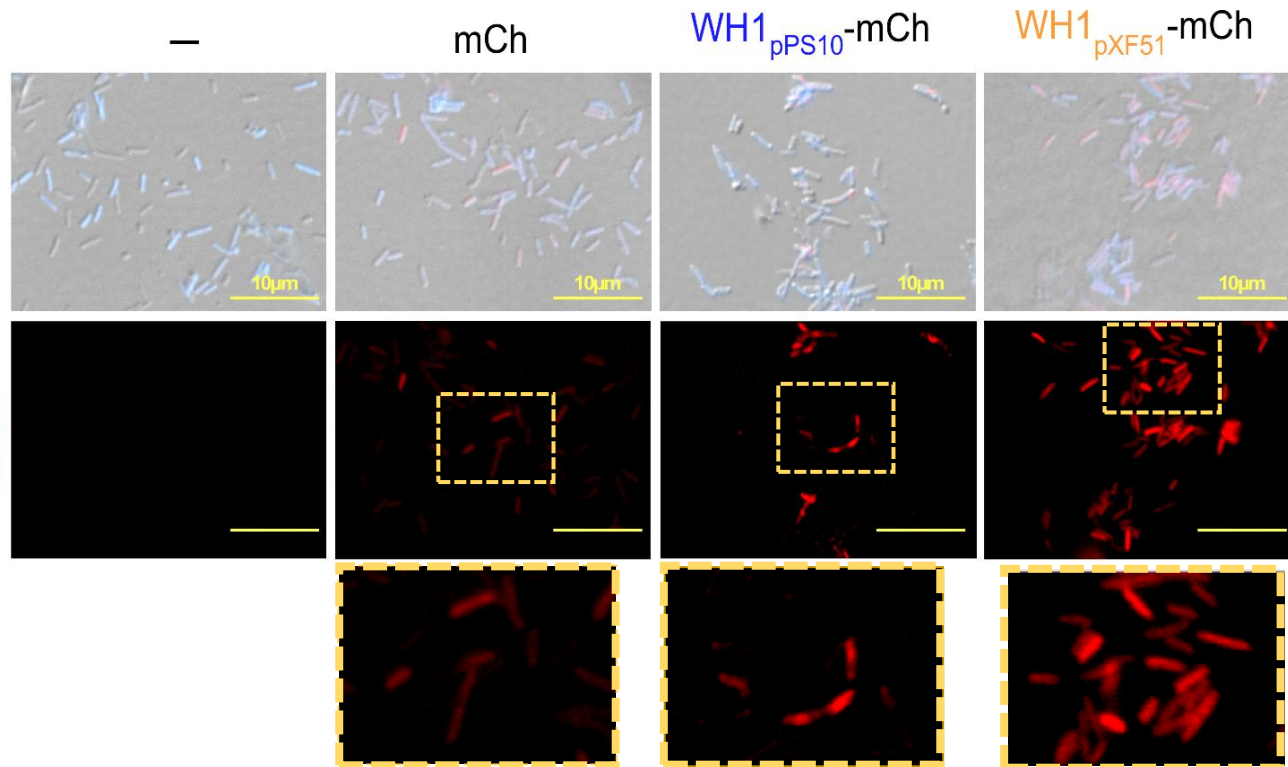
WH1(RepA)

- Amyloid aggregation
- Toxic for bacteria



X. fastidiosa ssp. *fastidiosa* IVIA5235 is hampered by the WH1 domains

DIC + merge



Rep-WH1 prions from the phytopathogen *Xylella fastidiosa* and their potential for controlling plant disease

Leticia Lucero-López¹, Blanca B. Landa², Rafael Giraldo¹

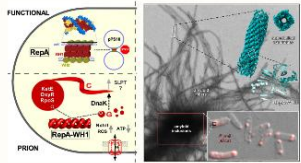
1. National Centre for Biotechnology (CNB-CSIC), Madrid, Spain.
2. Sustainable Agriculture Institute (IAS-CSIC), Córdoba, Spain.
llucero@cnb.csic.es

Introduction

Xylella fastidiosa (*X. fastidiosa*) is a phytopathogenic bacteria affecting economically relevant crops worldwide (vines, coffee, olive and fruit trees, among others). Its pathogenicity mainly relies on its overgrowth within the plant xylem, which restricts waterflow to the apical parts, withering the plant. Since no effective solution for its eradication has been provided to date, new strategies are currently being developed.

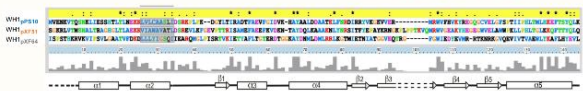
WH1_{pp810}, a protein domain belonging to the pPS10 plasmid replication protein RepA, from *Pseudomonas savastanoi*, has been proven to form amyloid aggregates that are harmful for *E. coli*; WH1_{pp810} interacts with the cell envelope, disrupting the proton-motive force, increasing intracellular ROS and depleting ATP.

Taking this into consideration, would there be one or several homologous proteins in *X. fastidiosa* which elicit similar toxicity similar effects?



RepA naturally forms the "handcuffing complexes" to avoid new plasmid replication rounds. These were proven to be of amyloid nature. The WH1 domain, fused to mCh, is able to form amyloid aggregates that are toxic for *E. coli* and that form fibrils in vitro.

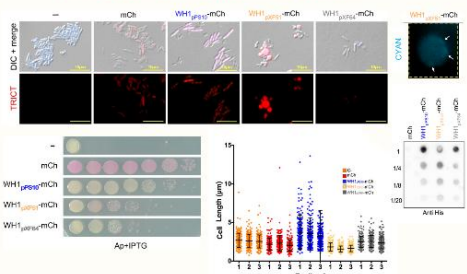
Homologues of WH1_{pp810} in *X. fastidiosa*



>70 Rep(WH1) *X. fastidiosa* sequences sharing >40% sequence identity with WH1_{pp810} were found in databases, of which two (WH1_{pp811} and WH1_{pp814}) were selected for downstream experiments. An amyloid stretch was found preserved in the three WH1 domains and high folding homology was detected using AmyloPred and AlphaFold2, respectively. The three proteins were fused to mCherry (mCh) for their detection using its red fluorescence.

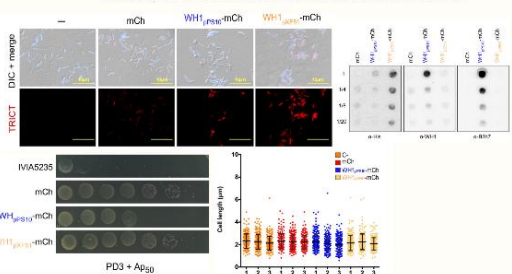


Toxicity of the three WH1 domains in *E. coli*



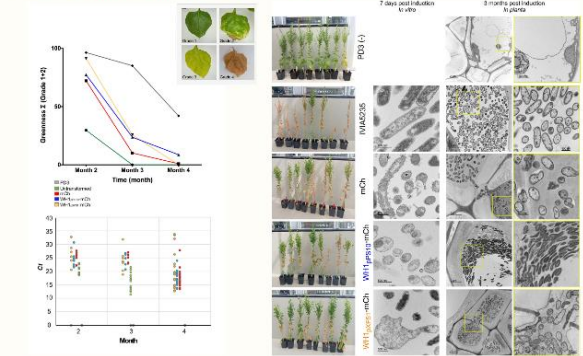
The three proteins produce toxicity to *E. coli* as shown in serial dilutions. WH1_{pp810}-mCh promotes cell filamentation and protein aggregation into solid aggregates, whereas WH1_{pp811}-mCh also produces solid aggregates which are mostly polar, but don't affect cell shape. WH1_{pp814}-mCh shifts cell shape into coccoid, despite no visible aggregates were detected in red fluorescence. However, dot blots revealed that aggregation is produced in all cases. Thioflavin-S (Th-S, cyan), a marker for amyloids, is positive in the three cases, also revealing solid aggregates in WH1_{pp814}-mCh-expressing cells.

Toxicity of the three WH1 domains in *X. fastidiosa*



WH1_{pp810}-mCh could not be expressed in *X. fastidiosa*, probably due to its toxicity. Both WH1_{pp811}-mCh and WH1_{pp814}-mCh were toxic for *X. fastidiosa*. WH1_{pp810}-mCh forms solid intracellular aggregates, and WH1_{pp811}-mCh looked diffuse. Despite Th-S stain could not be done to these cells, dot blots revealed protein aggregation in both cases, with amyloid traits for WH1_{pp810}-mCh (B3n7 antibody labelling). Results resemble those obtained in *E. coli*, excepting shifts in cell morphology, which were absent in *X. fastidiosa*.

Effects of WH1 to *X. fastidiosa* virulence to *N. benthamiana*



Whereas plants injected with control *X. fastidiosa* rapidly withered, the ones injected with *X. fastidiosa* expressing WH1_{pp810}-mCh or WH1_{pp811}-mCh experienced slower withering along time and higher qPCR Ct values, indicative of lower bacterial concentration. TEM images of cells cultured in vitro up to day 7 post-infection showed acute harm to cell envelopes when WH1_{pp810}-mCh and WH1_{pp811}-mCh were expressed. Section of petioles taken 3 months after bacteria injection into plants, showed chlorified harmful effects after expression of the same proteins.

Objectives

- Search for WH1_{pp810} homologues in *X. fastidiosa*.
- Analyze the effects of WH1 proteins in the model bacteria *E. coli* and in the phytopathogen *X. fastidiosa* (ssp. *fastidiosa* IMA5233).
- Assess whether these proteins have any effect on the virulence of *X. fastidiosa* to plants, using *Nicotiana benthamiana* (*N. benthamiana*) as a model.

Conclusions

- Two WH1_{pp810} homologues were found *in silico* in *X. fastidiosa*. Both were predicted similar folding and amyloidogenicity, to that of WH1_{pp810}.
- The three WH1 domains were proven harmful for *E. coli* and *X. fastidiosa*.
- The three proteins were proven amyloid in *E. coli*, whereas only WH1_{pp810}-mCh could be proved as an amyloid in *X. fastidiosa*.
- WH1_{pp810}-mCh and WH1_{pp811}-mCh hampered *X. fastidiosa* virulence to *N. benthamiana*.

References

- Giraldo R. (2024). PLoS Pathog. 2024. 20:e1012253
- Lucero-López L, Giraldo R. (2026). Submitted.
- Lucero-López L, Martín-Jouve B, Olivares C, Landa BB, Giraldo R. 2026. In preparation.

Poster
number
53



PID2021-124866OB-I00
PID2024-156842OB-I00



GENETIC DIVERSITY OF *XYLELLA FASTIDIOSA* IN THE OCCITANIA FRENCH REGION

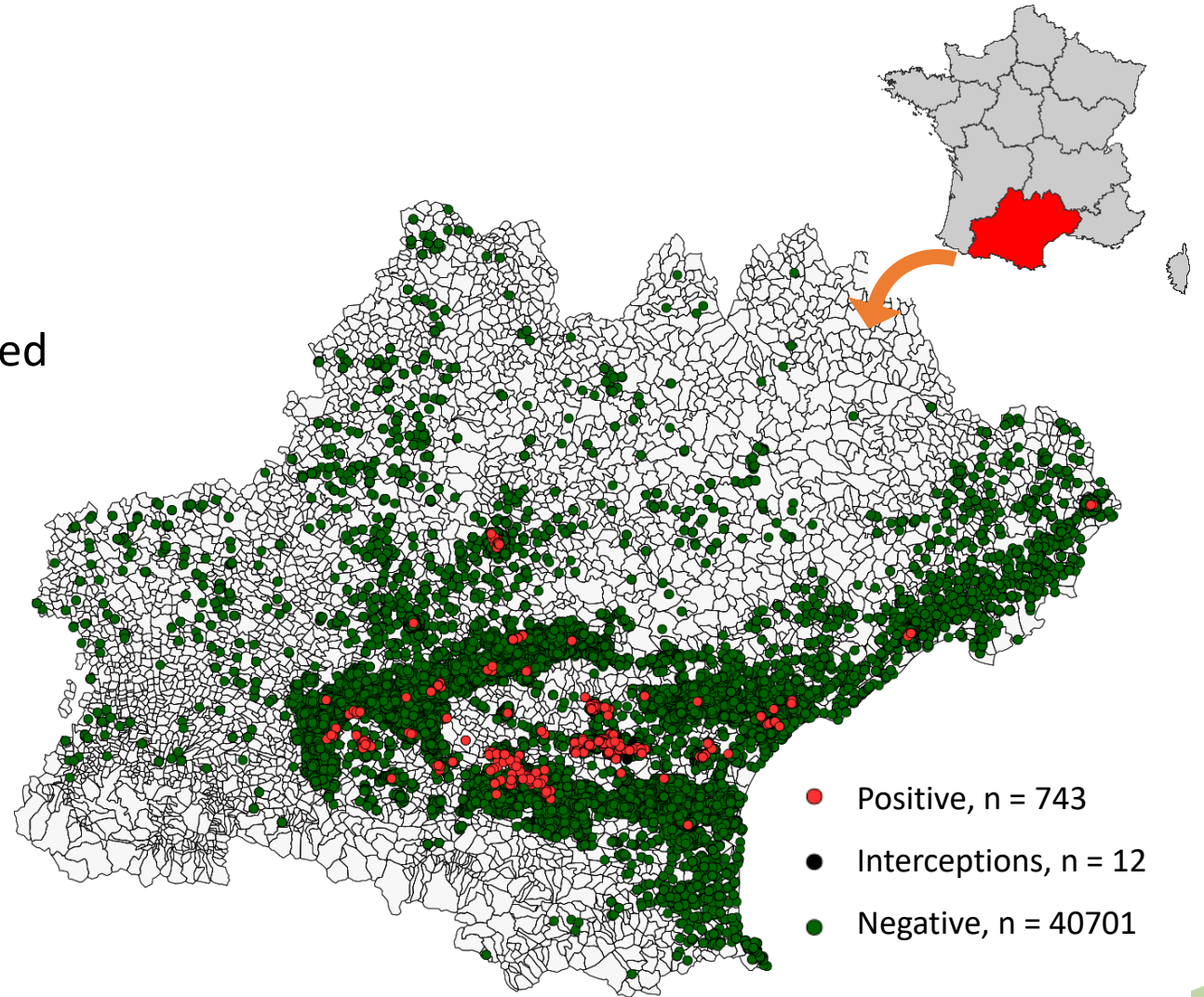
Victor Nelaton, PhD Student
ANSES Plant Health Laboratory



REGIONE PUGLIA

Why Occitania ?

- ❖ Most recent French region to be shown infected
- ❖ No knowledge of the origin of contamination
- ❖ A large area covered by the bacterium



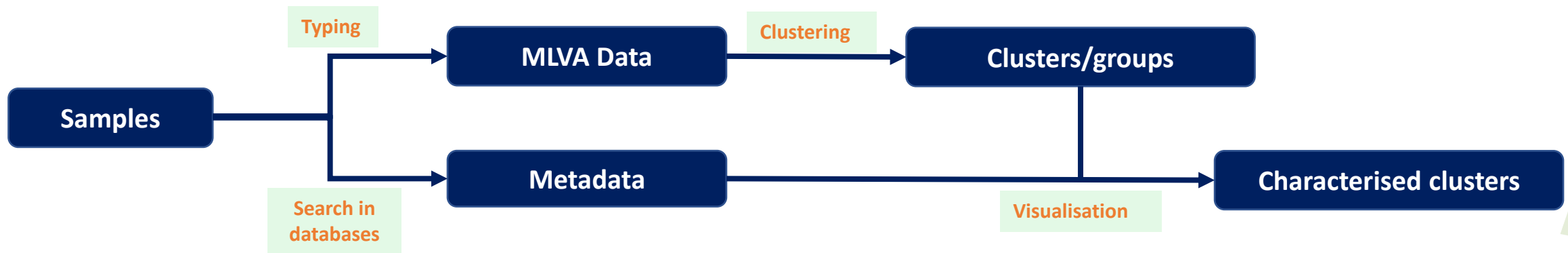
Occitania's *X. fastidiosa* surveillance sampling, 01/01/2015 – 12/03/2026
(Shiny Anses)

Objectives

- ❖ Confirm the presence of ST6 and ST7 only
- ❖ Look for relativeness with previously known strains (French and/or Spanish)
- ❖ Highlight the fine intra-regional genetic structuration and possible gene flow

Method

- ❖ MLVA (VNTR-12 scheme) typing of 331 samples collected during official surveillance
- ❖ Genetic clustering
- ❖ Visualisation and characterisation of clusters using metadata



Results

See Poster !

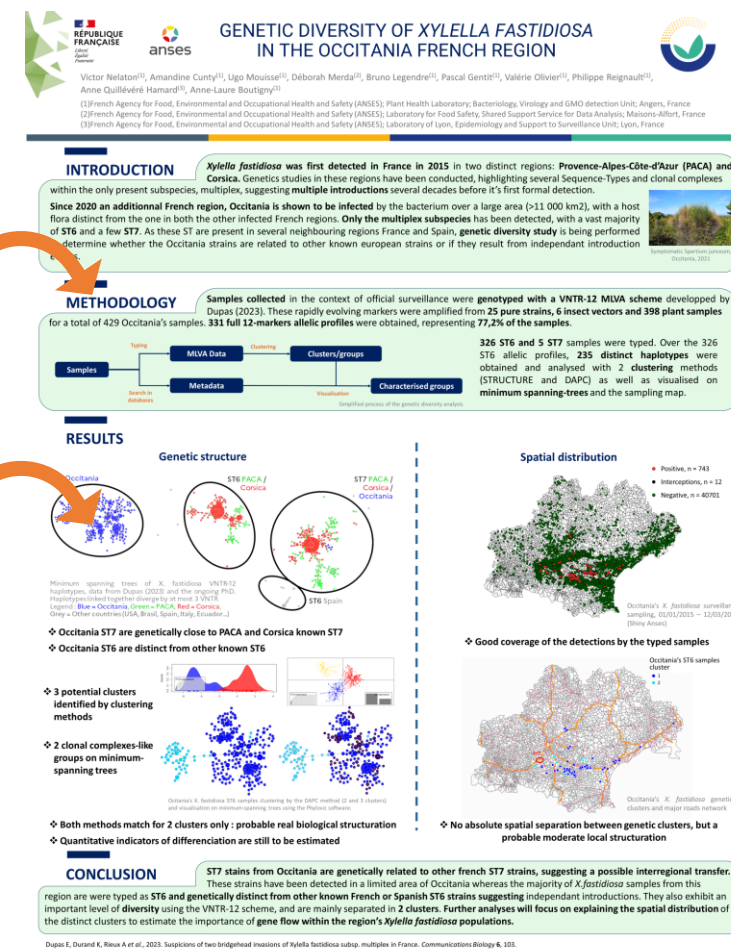
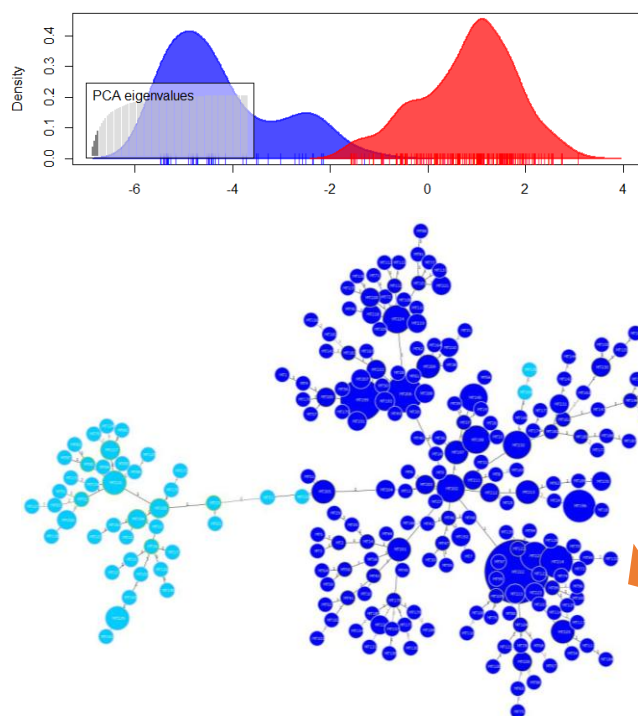
326 ST6 strains and 5 ST7 strains

The ST7 population is genetically similar to other French ST7 strains

ST6 strains are a new cluster: probable independent introduction in the region

Two subclusters within ST6 Occitania strains: or distinct introductions or diversification?

DAPC scatterplot and minimum-spanning tree of ST6 Occitania strains. Colors = DAPC clusters





Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026



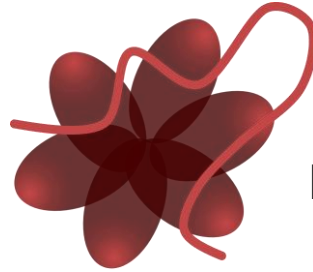
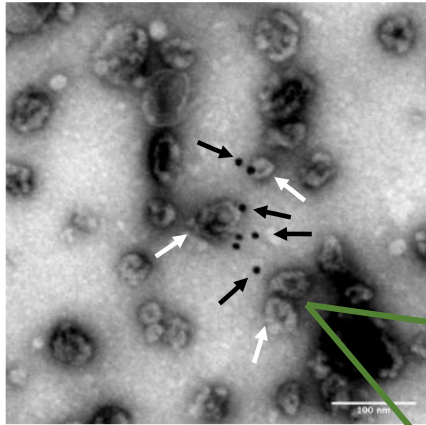
REGIONE PUGLIA

Xylella fastidiosa and its Extracellular Vesicles

Xylella fastidiosa (Xf);

- can switch between **planktonic growth and biofilm** in response to environmental cues, mediated by Quorum Sensing (QS).
- releases significantly higher extracellular vesicles (Xf-EVs) than closely related species¹.
- Identified RNA chaperone **Hfq** and sRNAs as cargo of Xf-EVs².

α -Hfq



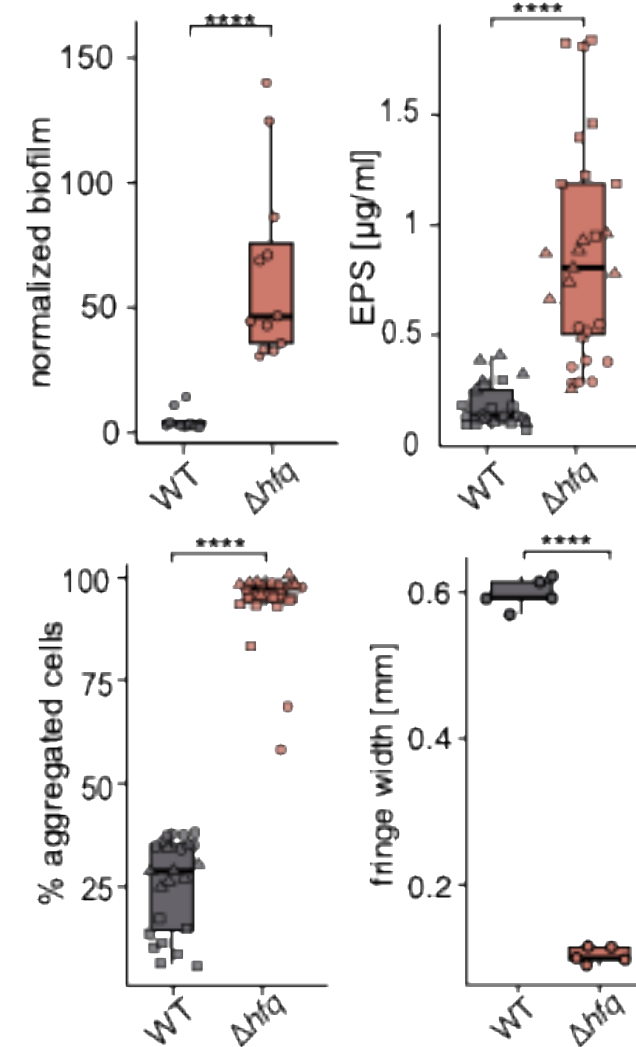
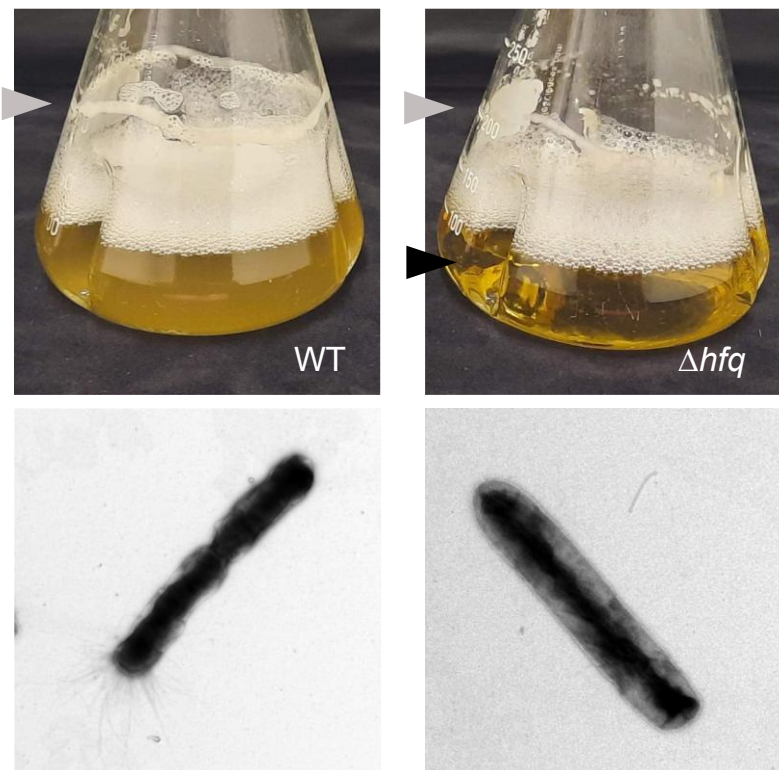
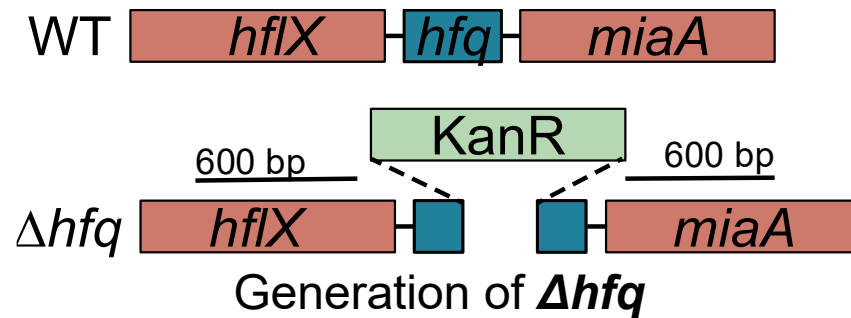
Hfq – central regulator of RNA metabolism
facilitates **base pairing** between **sRNA** and **mRNAs**

What are the roles of Xf-EVs and Hfq in Xf ?

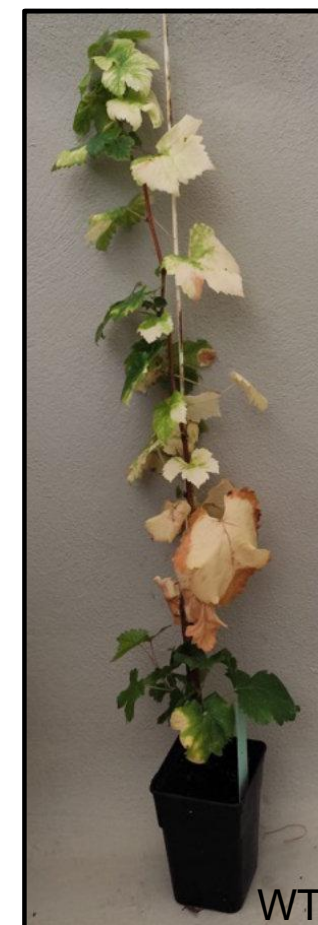
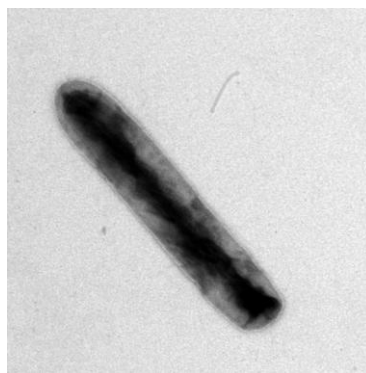
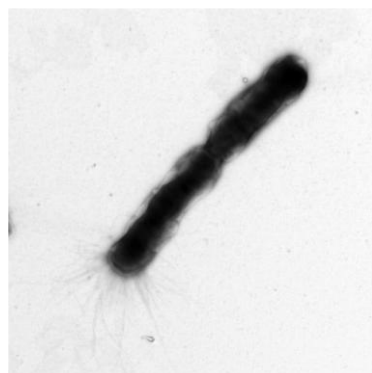
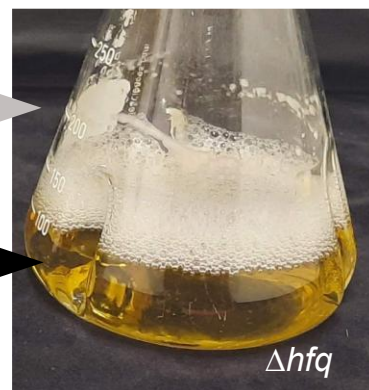
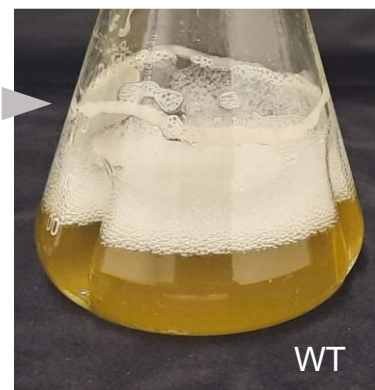
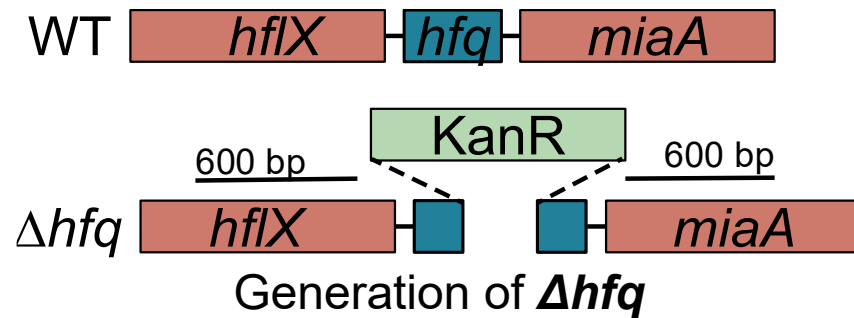
¹ Ionescu et al., 2014 *PNAS*

² Ruf et al., 2025 *JEV*

Hfq mediates aggregation, biofilm regulation and motility

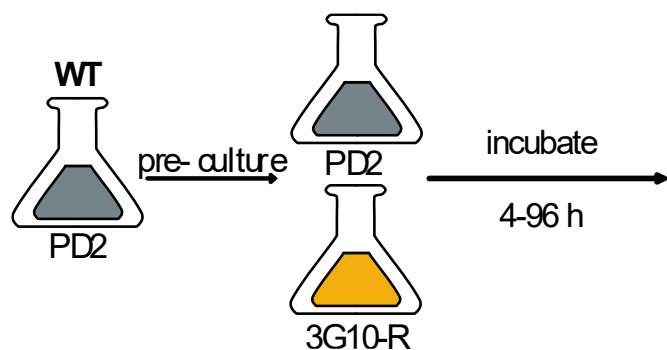


Hfq mediates aggregation, biofilm regulation and motility



Grapevine cultivar
Sauvignon Blanc

Hfq facilitates transcriptional regulation via small RNAs



Transcriptomics

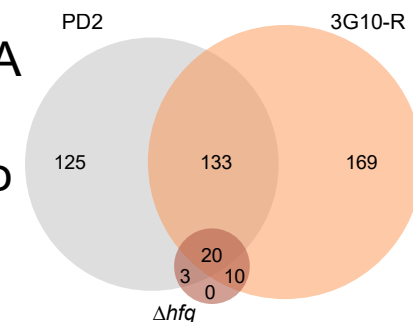
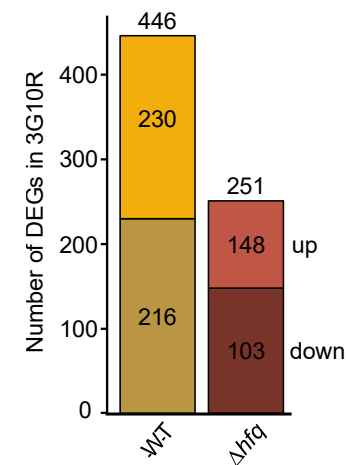
Differentially expressed genes as sudden response

Type 4 pilus, transcriptional regulator and signaling responses are impaired in *Δhfq*

sRNA Sequencing

De novo sRNA annotation

Novel Hfq-dependent sRNA candidates to mediate the gene expression relating to biofilm, QS system, virulence



PD2 : nutrient rich medium

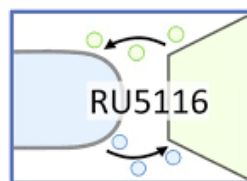
3G10R : xylem-mimicking, nutrient poor medium



Poster #73

Hfq-dependent small RNAs integrate environmental sensing with lifestyle transitions and virulence in *Xylella fastidiosa*

Alessa Ruf, Elif Olkun, Marcel Sprenger, Jingli Lao, Miguel Román-Écija, Vincent Ohlhauser, Neysa Rodriguez, Marta Martin-Rivero, Andreas Klingl, Blanca Landa, Kai Papenfort, and Silke Robatzek #



DFG Research Unit
"exRNA"



"MultiX"



REGIONE PUGLIA