

A LONG-TERM STUDY OF OLIVE CULTIVARS DEMONSTRATES A WIDE RANGE OF RESPONSES TO *XYLELLA* *FASTIDIOSA* SUBSP. *PAUCA* ST53

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Response to Xfp in olive:

XF: $>10^5$ CFU/ml
Severe symptoms

XF: $<10^4$ CFU/ml
Mild symptoms

Highly susceptible

Resistant



Screening olive cultivars to Xfp:

PHENOTYPIC ASSESSMENT: symptoms YES/NO

major challenges

UNDER CONTROLLED CONDITIONS



- Inconsistency in the efficiency of Xf mechanical inoculation (**vector-mediate transmission more efficient**)
- Bacterial load can reach high titer also in resistant small plants
- Long incubation period and staggered appearance of symptoms



Symptoms ► susceptible
No symptoms ► Resistant?



Screening olive cultivars to Xfp: **major challenges**

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No symptoms ► Resistant?

Screening olive cultivars to Xfp –field experiments

2015



Pilot project NP.EFSA.ALPHA.2014.07



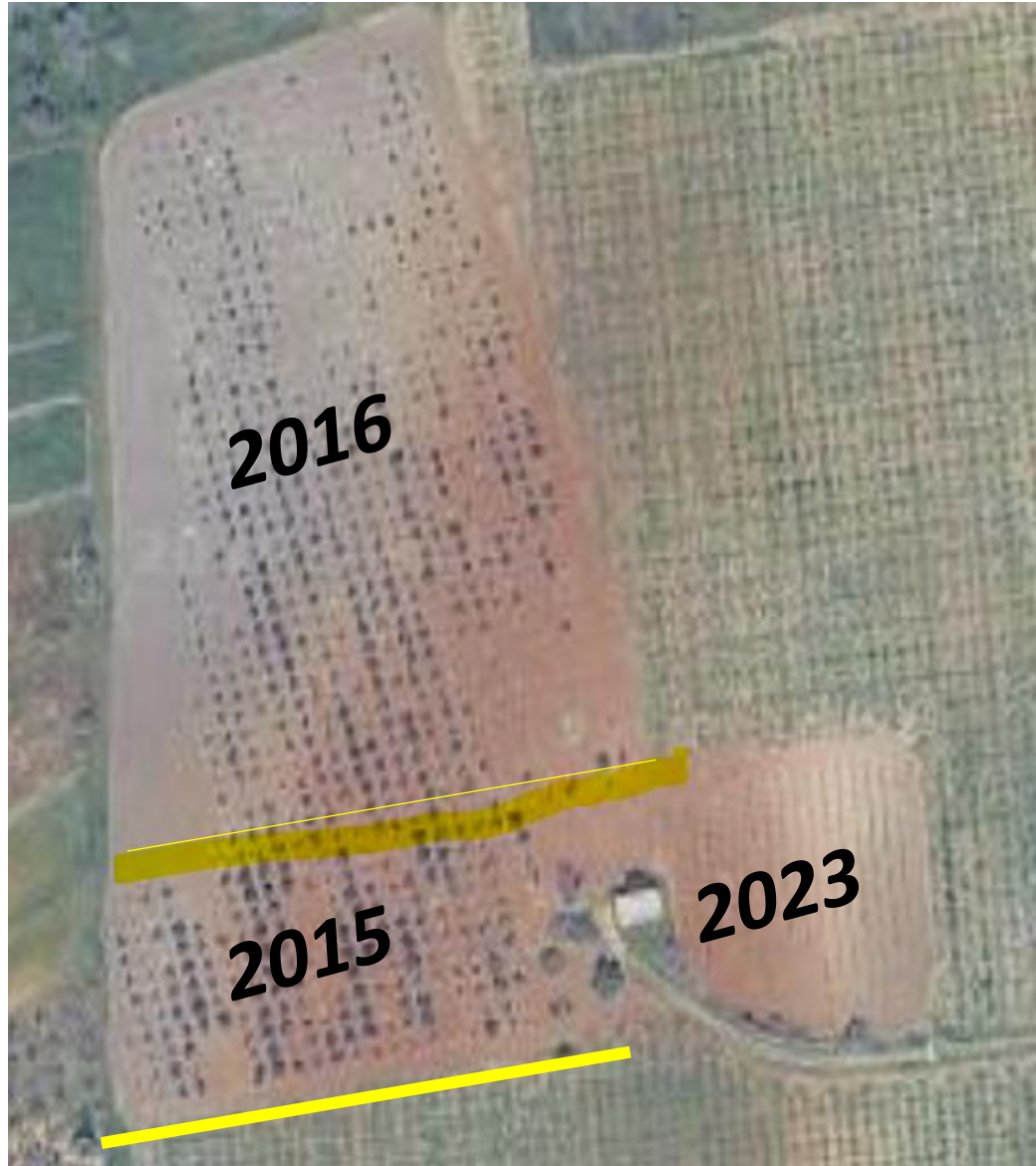
Centro di Ricerca
Sperimentazione e Formazione
in Agricoltura "BASILE CARAMIA"

24 replicates

10 cultivars



Screening olive cultivars to Xfp –field experiments



National projects



ID plot	N. of cultivars/ selections	Total number of trees	Year of plantation	Location
Parcel A	10	144	2015	Parabita - Lecce
Parcel B	25	365	2016 (April)	
Parcel C	15	144	2016 (November)	
Parcel D	41	303	2023	
Plot E	<u>283</u>	820	2018-2022	Ugento – Lecce
Plot F	28	140	2025	Restinco- Brindisi
Total	<u>402</u>	<u>1916</u>		

74 cultivars – represented by a single trees



Parameters

CLASS	Qualitative assessment	Score of the symptoms (0-5)	Bacterial load CFU/ml	% prevalence infections
1	Highly susceptible	3,5-4	$>10^5$ (<25)	100
2	Susceptible	2-3		>80
3	Moderately susceptible	1-1,5	10^5 - 10^4 (25>Cq<28)	>50
4	Tolerant	0,5-1		<50
5	Resistant	0-0,5	$<10^3$	<50
6	Highly resistant	0	(Cq>28)	<50

Cultivar	N. of trees*	%positive trees	Estimated bacterial population		N. of symptomatic trees	Highest score for symptoms	Phenotypic category (Class)
			CFU/ml	Average Cq value			
Leccino	24,00	▶ 41,67	8,36E+03	28,57	6	0,5	6
<u>FS17</u>	▶ 3,00	100,00	4,21E+04	26,24	1	▶ 0,5	5
Frantoio	22,00	90,91	3,71E+04	26,42	16	1-1,5	3-4
Cima di Melfi	21,00	100,00	3,19E+05	23,32	21	2,5	2
<u>Don Carlo</u>	▶ 3,00	100,00	1,29E+05	24,62	2	▶ 2,5	2
Koroneiki	22,00	95,45	4,70E+04	26,08	21	2,5	2
Arbequina	21,00	100,00	7,12E+04	25,48	20	4	1
Cellina di Nardò	23,00	100,00	3,61E+05	23,14	23	4	1
Coratina	22,00	100,00	2,73E+05	23,54	20	4	1
Arbosana	24,00	100,00	3,49E+05	23,19	24	3	1

Cultivar	%positive trees	Estimated bacterial population		N. of symptomatic trees	Highest score for symptoms	Phenotypic category (Class)
		CFU/ml	Average Cq value			
Leccino	▶ 38,46	5,22E+03	29,25	2	0,2-0,5	6
Cipressino	{ 73,91	1,74E+04	27,51	4	0,5	5
Ascolana Tenera		1,98E+04	27,33	8	0,5	5
Toscanina		2,43E+05	23,71	8	1	4
Bella di spagna	86,36	2,22E+05	23,84	18	1,5-2	3
Picholine						
Languedoc	66,67	5,63E+04	25,82	8	1,5-2	3
Nociara	93,33	2,28E+05	23,8	14	1,5-2	3
Oliastro	94,74	2,91E+05	23,45	10	1,5	3
Picholine	92,31	2,77E+04	26,84	8	1,5-2	3
Termite di Bitetto	81,82	4,70E+04	26,08	8	1,5	3
Carolea	86,67	1,68E+05	24,24	11	2,5-3	2
Koroneiki	100,00	8,77E+04	25,18	18	3	2
Peranzana	86,67	4,63E+05	22,78	5	3	2
Nolca	100,00	3,66E+05	23,12	10	4,5-5	1
Ogliarola salentina	100,00	1,97E+05	24,01	23	3,5-4	1
Simone	100,00	9,80E+04	25,02	18	3,5-4	1

Tollerant

Infected and symptomatic Cellina di Nardò trees



Leccino trees



Leccino



Ascolana tenera



Leccino



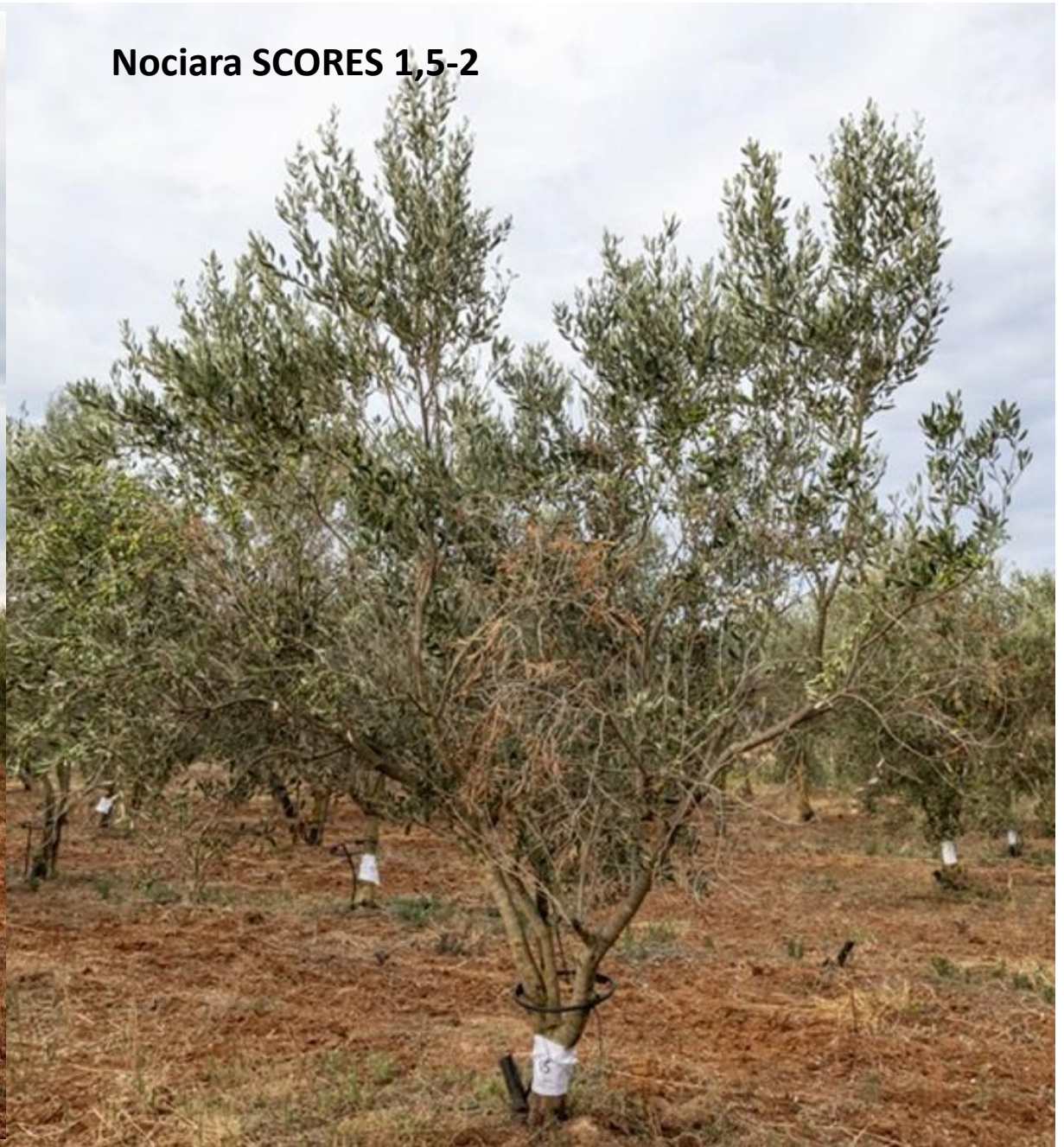
Cipressino



Cellina di Nardò (control) – SCORES 4



Nociara SCORES 1,5-2



Ogliarola (control) – SCORES 3-4



Picholine SCORES 1,5-2



Cellina di Nardò 2015



Ogliarola 2016



Infected and symptomatic Koroneiki trees – all productive - Arbequina & Arbosana (similar)



Cultivar	%positive trees	Estimated bacterial population		Severity of symptoms	Phenotypic category (Class)
		CFU/ml	Average Cq value		
Mari	12,50	2,38E+03	30,38	-	6
Uovo di Piccione	0,00	3,31E+03	29,91	-	6
FS17	0,00			-	6
Gordal Sevillana	0,00			-	6
Medjhol	0,00			-	6
Memeçik	20,00			-	6
Myrtolia	0,00			-	6
Ascolana Tenera	22,22	7,86E+03	28,66	-	5
Grappolo	66,67	2,50E+04	26,99	-	5
Coratina	70,00	3,54E+04	26,49	-	5
Leccino	23,53	7,69E+03	28,69	-	5
Leccio del Corno	33,33	6,51E+04	25,61	Mild	4
Frantoio	50,00	1,18E+04	28,07	Mild	4
Giulia	75,00	2,89E+04	26,78	-	4

Koroneiki	44,44	3,54E+04	26,49	Medium	2
Manzallinera do Huerca	100,00	2,33E+05	23,77	Medium	2
Hojiblanca	54,55	1,00E+05	24,99	Medium	2
Picudo	80,00	6,92E+05	22,2	Medium	2
Arbosana	100,00	2,93E+05	23,44	Intense	1
Barnea	85,71	3,08E+05	23,37	Intense	1
Llumeta	100,00	3,89E+05	23,03	Intense	1
Manzanilla de Sevilla	70,00	5,95E+05	22,42	Intense	1
Mastoidis	100,00	8,12E+05	21,97	Intense	1
Meski	60,00	1,28E+05	24,64	Intense	1
Picholine marroccaine	77,78	3,10E+05	23,36	Intense	1
Pinonera	87,50	9,27E+04	25,1	Intense	1
Cellina di Nardò	100,00	7,27E+04	25,45	Intense	1

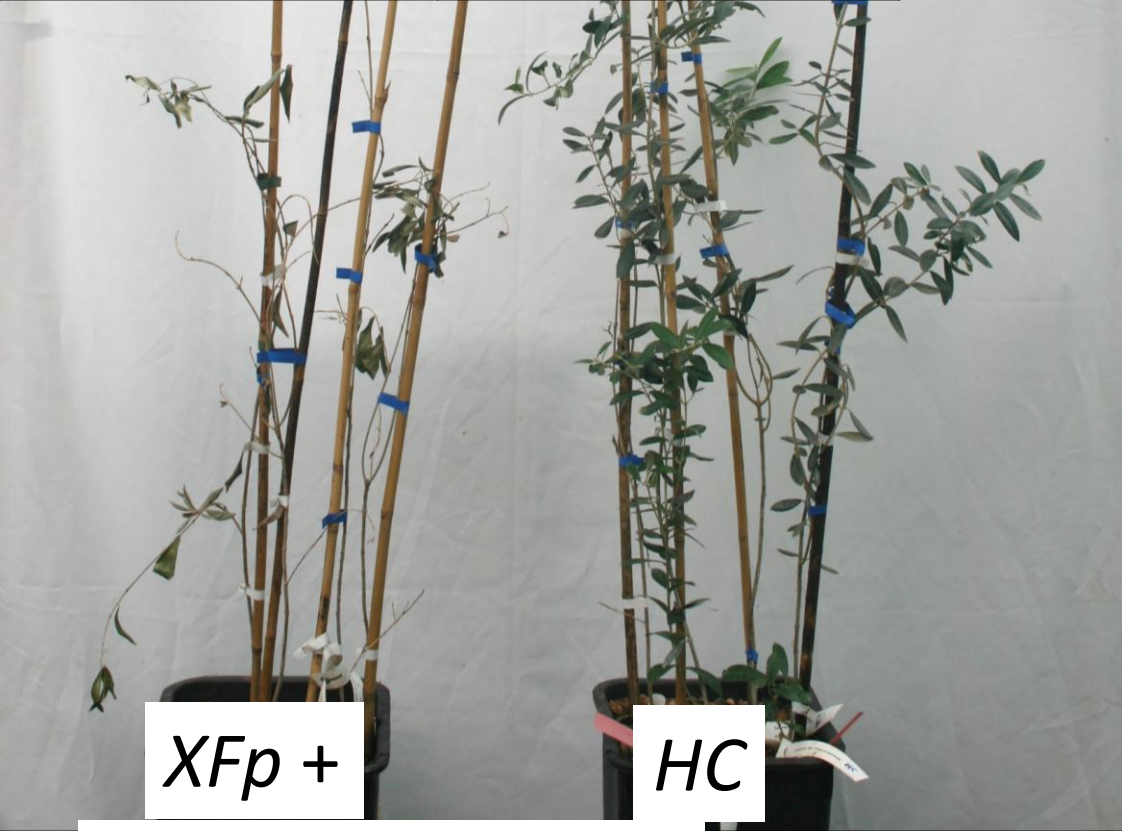
Screening olive cultivars to Xfp: recent plantation (2023)

Cultivar	% positive trees	Estimated bacterial population		Appearance of initial sign of symptoms (Yes/no)	Phenotypic category (Class)
		Average Cq value	CFU/ml		
Ghiacciolo	66,67	22,92	4,20E+05	yes	1
Koroneiki	74,29	21,28	1,31E+06	yes	1
Nebbia del Menocchia	100,00	21,36	1,24E+06	no	1
Nostrale di Rigali	27,27	22,36	6,20E+05	yes	1
Raggiola	81,82	24,57	1,34E+05	yes	1
Razzo	83,33	23,37	3,08E+05	yes	1
Rossellino Cerretano	50,00	22,4	6,03E+05	yes	1

UOVO DI PICCIONE	class	5
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Coratina plot 1	class	2
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Coratina plot 2	class	6
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GREENHOUSE TEST

Conflicting data

GIULIA	class	4
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COMMERCIAL OLIVE GROVE

Overall preliminary characterization

CLASS	Qualitative assessment	% Cultivars
1	Highly susceptible	46%
2	Susceptible	
3	Moderately susceptible	35%
4	Tolerant	
5	Resistant	19%
6	Highly resistant	



Feature of the Resistant cultivars



Leccino

ID sample	qPCR Stem	> qPCR Leaves
1	25,02	29,21
2	30,57	Neg
3	26,37	Neg
4	29,33	Neg
5	27,08	Neg
7	27,06	Neg
13	25,43	Neg
14	26,73	Neg
15	27,80	Neg
17	25,28	32,19
18	24,06	29,21
47	28,55	Neg
54	24,24	Neg
57	24,23	29,54

Cipressino

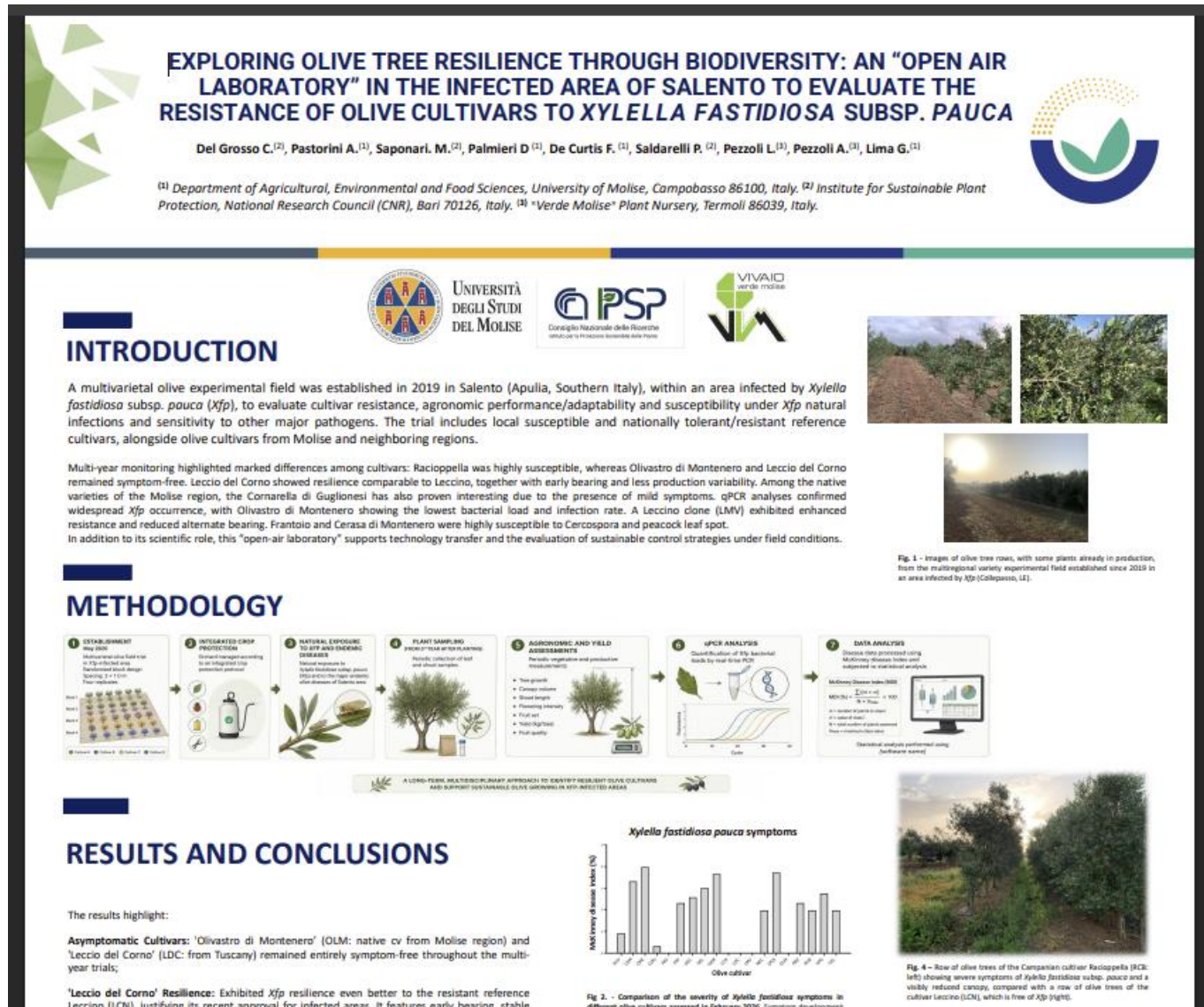
stem	> Leaves
25,02	29,11
23,75	30,28
20,55	25,75
26,04	26,15
25,97	26,54
22,11	21,22
23,11	25,02
23,28	Neg
23,24	24,11
22,75	27,99
22,75	23,32
27,29	Neg
20,84	22,16
24,28	26,08

Ascolana tenera

stem	> Leaves
25,17	Neg
23,97	27,01
22,73	33,11
28,76	Neg
25,20	25,54
23,28	Neg
22,42	Neg
29,16	27,32
21,36	19,14
29,29	Neg
24,55	23,61
24,22	26,39
23,35	21,34

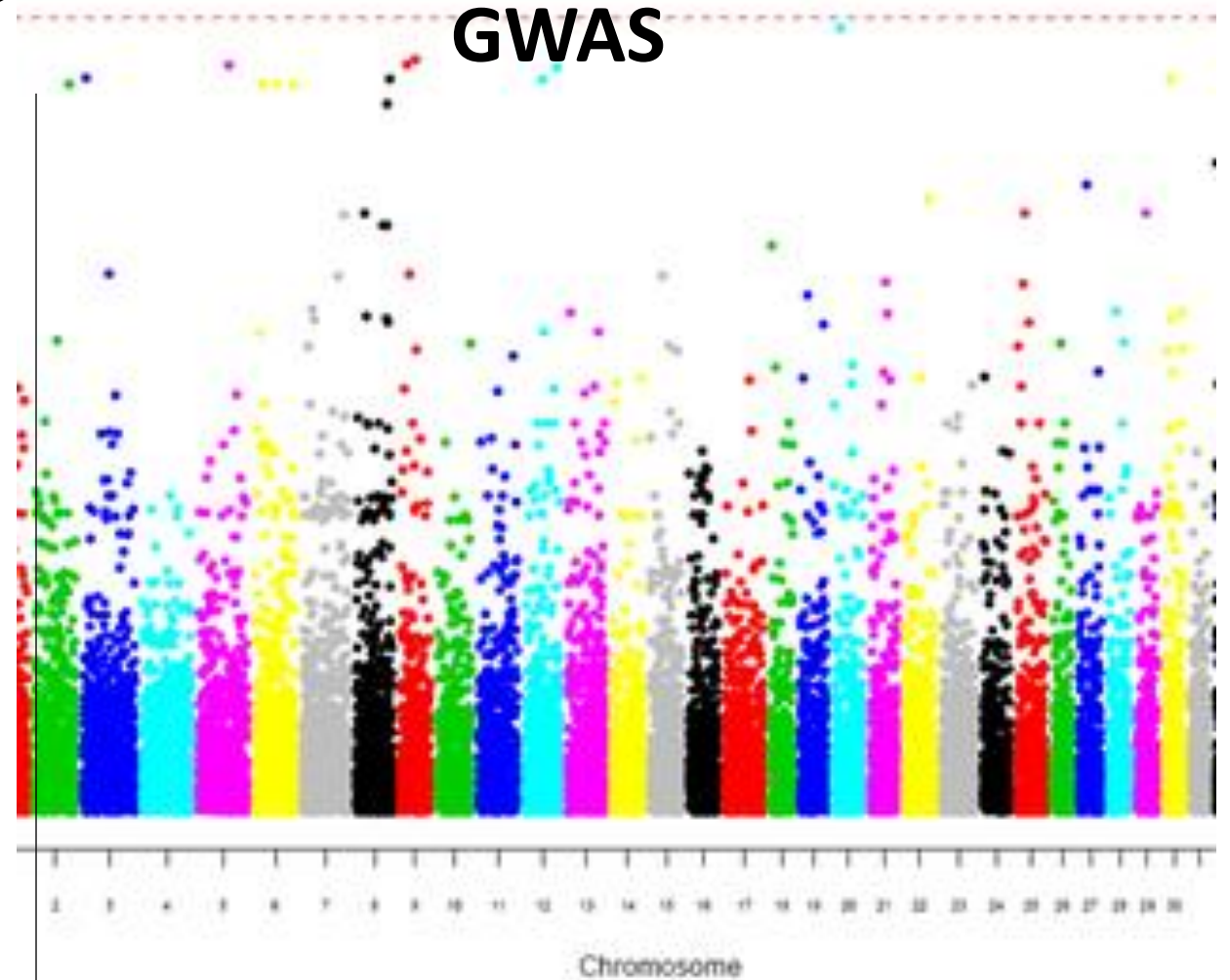
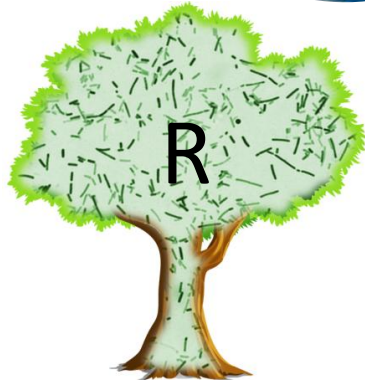
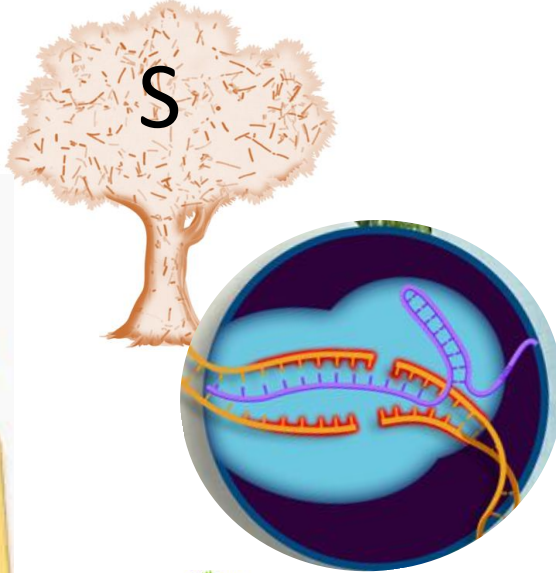
ADDITIONAL DATA

POSTER N. 16



Phenotyping & genetics

Classification of a large panel of olive cultivars



MINISTERO DELL'AGRICOLTURA
DELLA SOVRANITÀ ALIMENTARE
E DELLE FORESTE






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Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026



REGIONE PUGLIA

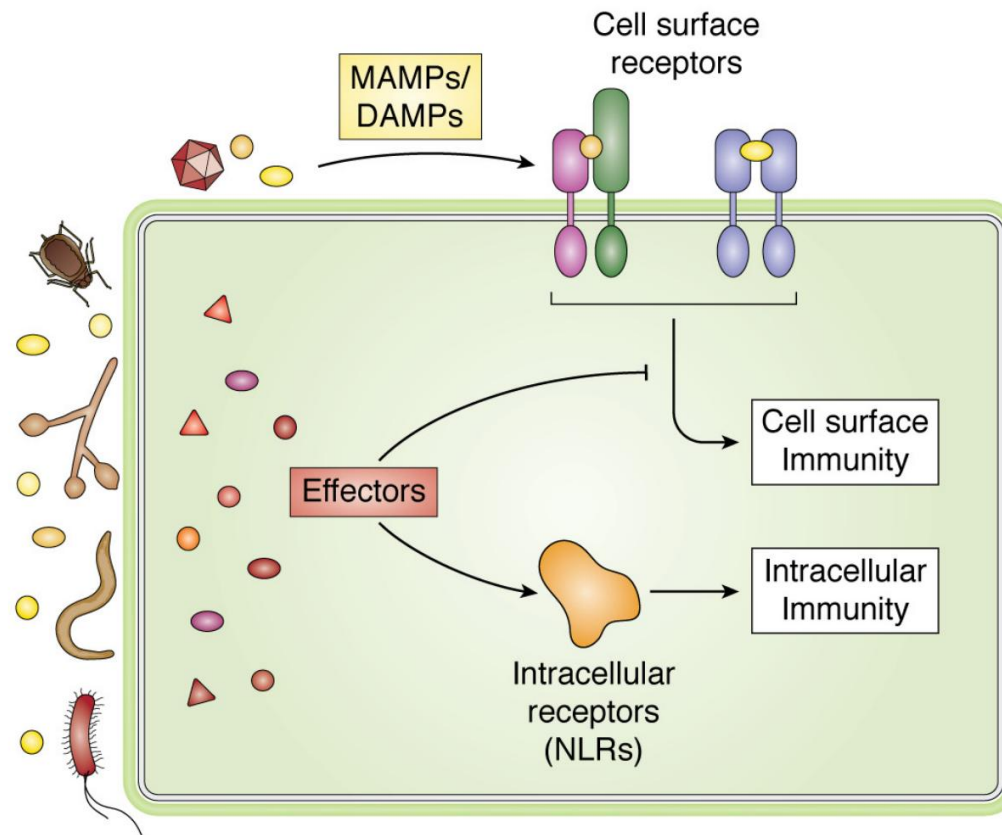
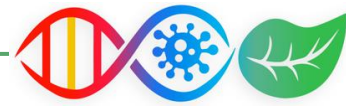


Genomic and molecular insights into olive immunity to *X. fastidiosa*: comparative analysis of Apulian cultivars Ogliarola salentina and Leccino



Plant-microbe interactions
Silke Robatzek





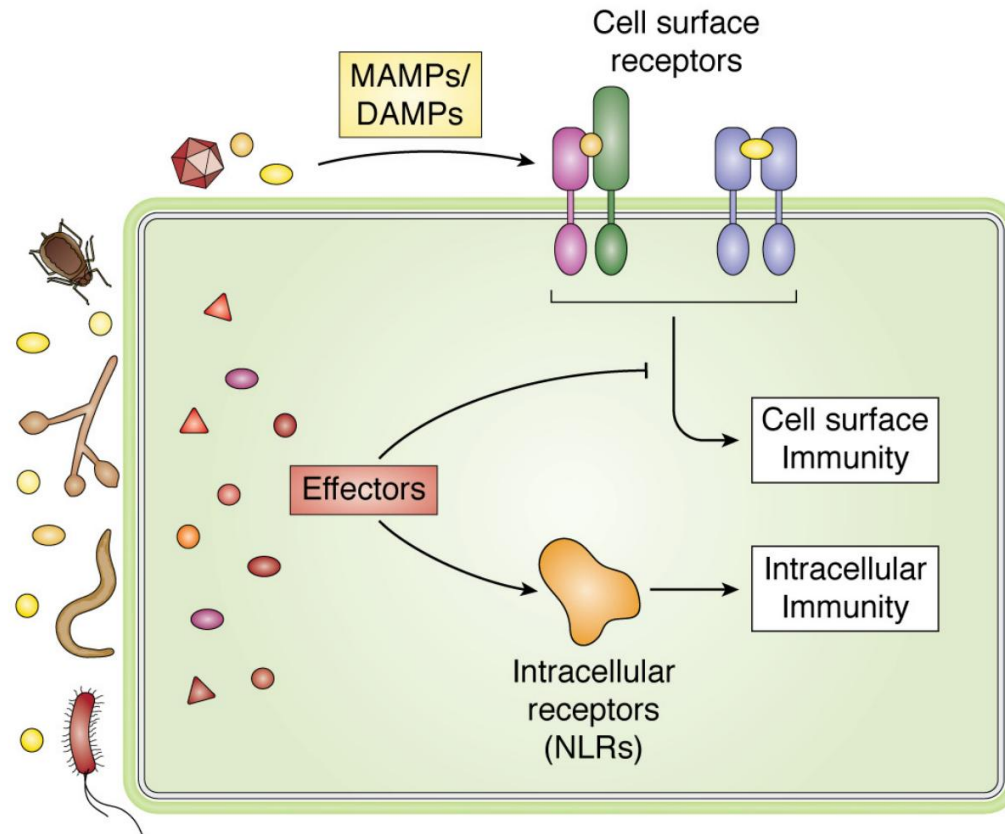
PRR immune signalling

Recognition of MAMPs and DAMPs

NLR immune signalling

Recognition of effectors

Introduction > The two tiers of the plant immune system



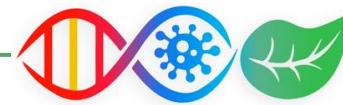
MAMPs/DAMPs

bacteria: flagellin (flg22, flgII-28)
cold-shock protein (csp22)
fungi: chitin
self/non-self: scoop + microbial scoop-like

PRRs

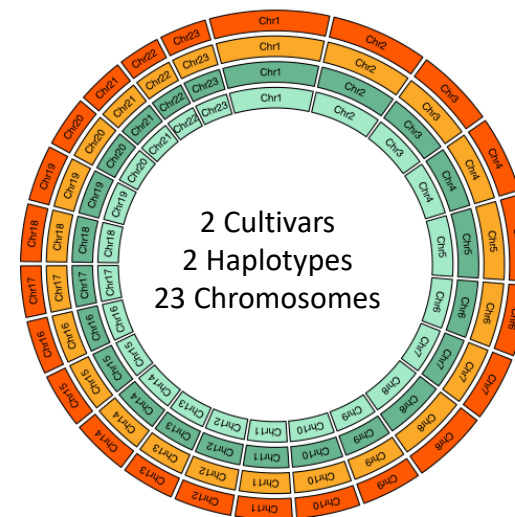
FLS2: flg22; FLS3: flgII-28
CORE: csp22
CERK1: chitin
MIK2: scoop + microbial scoop-like

Research focus > Olive cultivar susceptibility and resistance



resistant cultivar
Leccino

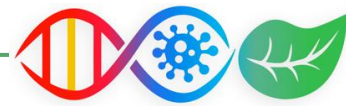
susceptible cultivar
Ogliarola salentina



- Ogliarola haplotype 1
- Ogliarola haplotype 2
- Leccino haplotype 1
- Leccino haplotype 2

Assembly	Assembly size (Mb)	Expected coverage (fold)	BUSCO completeness (% single / % duplicated)	Nb of genes
Ogliarola Hap 1	1211	26,7	83.8 / 13	41285
Ogliarola Hap 2	1414	26,7	84.3 / 12.4	40798
Leccino Hap 1	1412	20,2	83.4 / 13.1	40921
Leccino Hap 2	1162	20,2	83.3 / 13	40991

Research focus > Olive cultivar susceptibility and resistance



resistant cultivar
Leccino



susceptible cultivar
Ogliarola salentina

			Leccino		Ogliarola salentina	
			Hap 1	Hap 2	Hap 1	Hap 2
Nb of genes			40921	40991	41285	40798
Nb of PRRs	Nb of RLKs	LRR-RLKs	296	298	299	287
		LRR-RLKs Class XII	90	85	72	69
		LysM-RLKs	21	25	25	24
	Nb of RLPs	LRR-RLPs	101	129	99	116
		LysM-RLPs	20	24	21	18
	Nb of NLRs		CNLs	91	77	89
TNLs			6	10	8	9
RNLs			4	10	6	10
ARM-NLs			10	18	14	18
NLs			138	145	141	131

Research focus > Phylogenetic tree of olive RLK-XII



Species

- A_thaliana
- B_rapa
- Leccino_hap1
- Leccino_hap2
- M_truncatula
- N_benthamiana
- O_sativa
- Ogliarola_hap1
- Ogliarola_hap2
- S_lycopersicum
- S_moellendorffii
- S_pampasense
- S_raphanifolium
- S_tuberosum

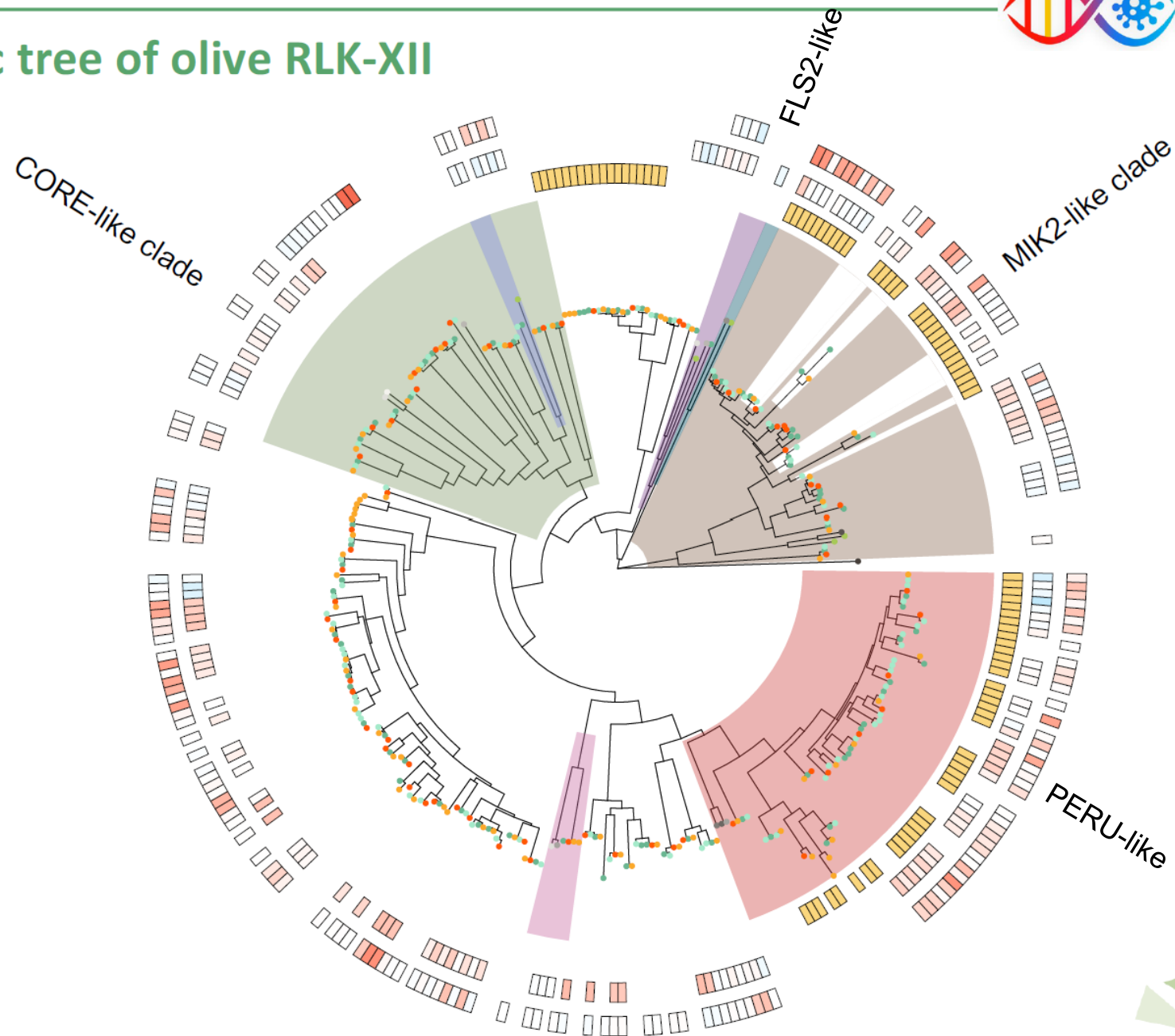
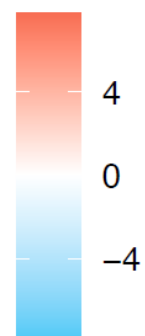
Clades

- CORE-like
- EFR-like
- FLS2-like
- FLS3-like
- MIK2-like
- NUT-like
- PERU-like

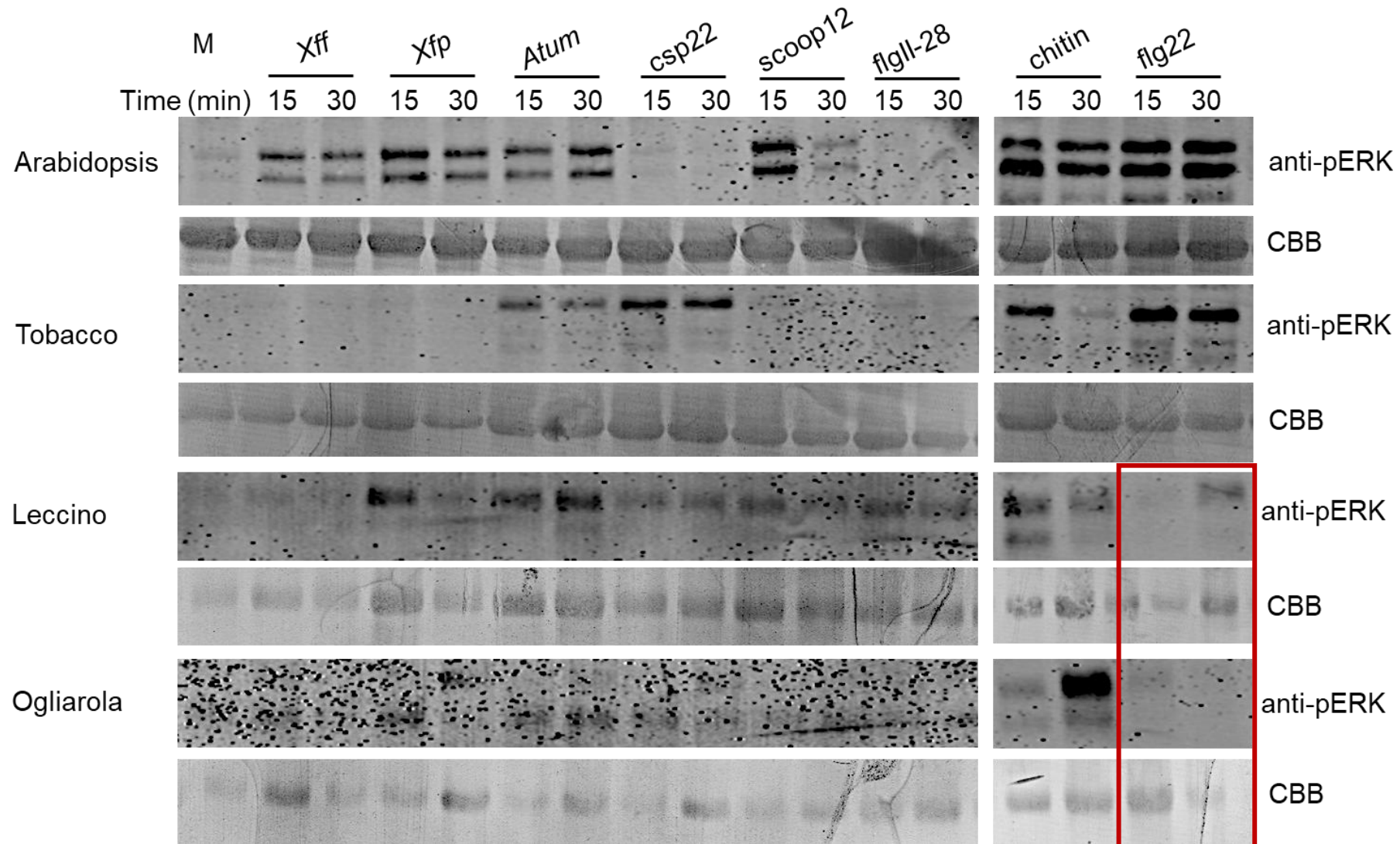
Chr16

- Chr16

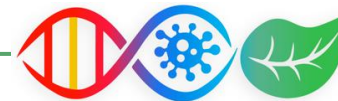
log2FC



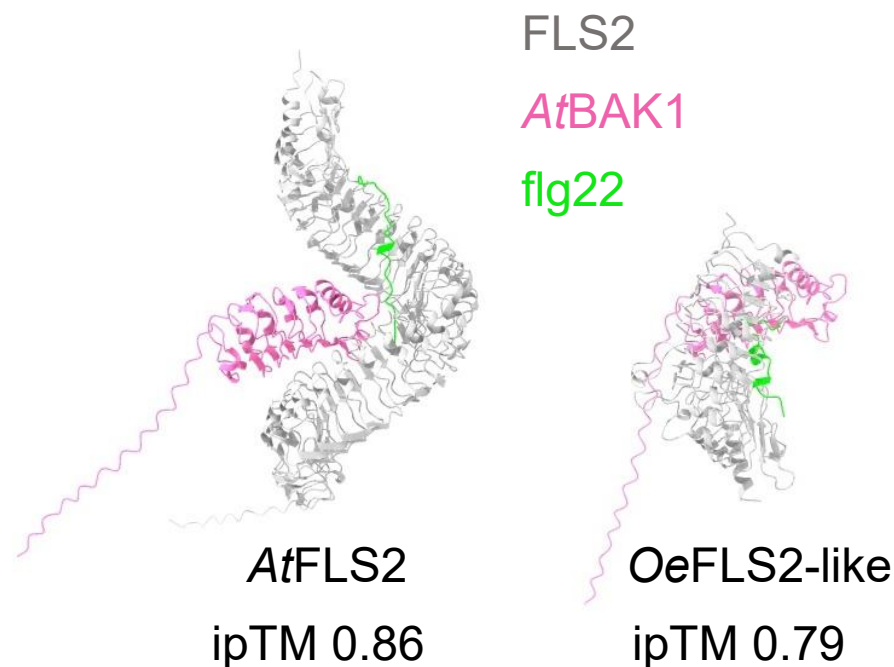
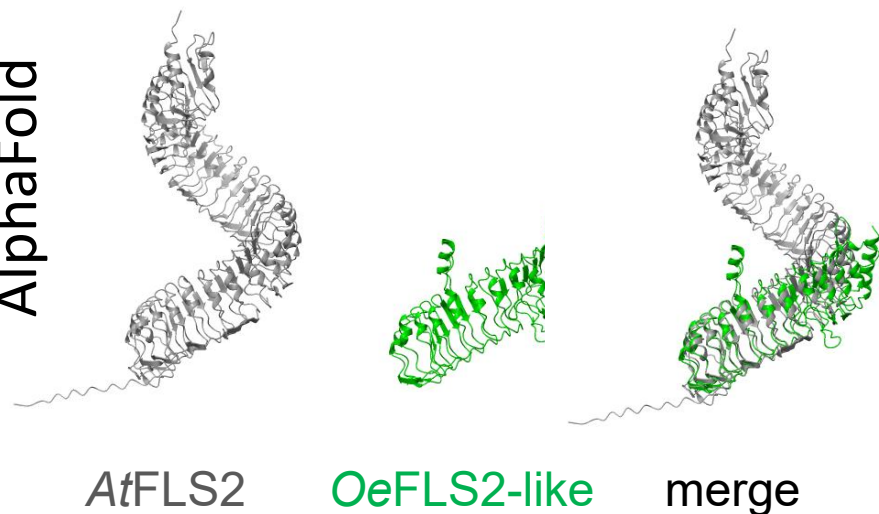
Research focus > MAPK activation induced by microbial patterns and bacterial extracts



Research focus > Analysis of *OeFLS2*-like from Leccino



AlphaFold



mamp-ml

	<i>flg22^{Pa}</i>	<i>flg15^{Pa}</i>	<i>flg15^{Ec}</i>	<i>flg22^{At}</i>	<i>flgII-28</i>
<i>OeFLS2</i>	1	1	1	1	0
<i>AtFLS2</i>	0	2	2	1	1

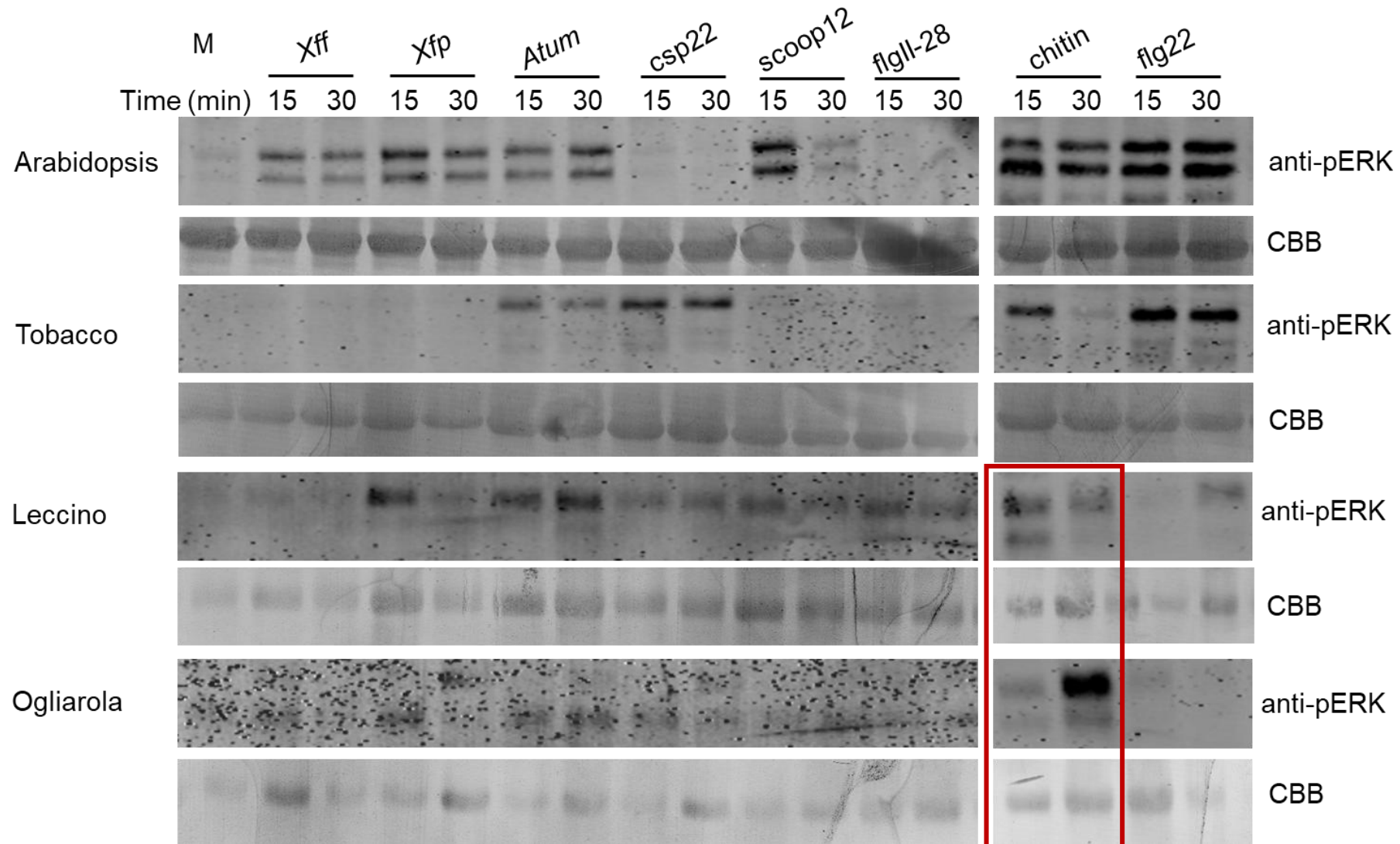
Outcomes:

0 = immunogenic

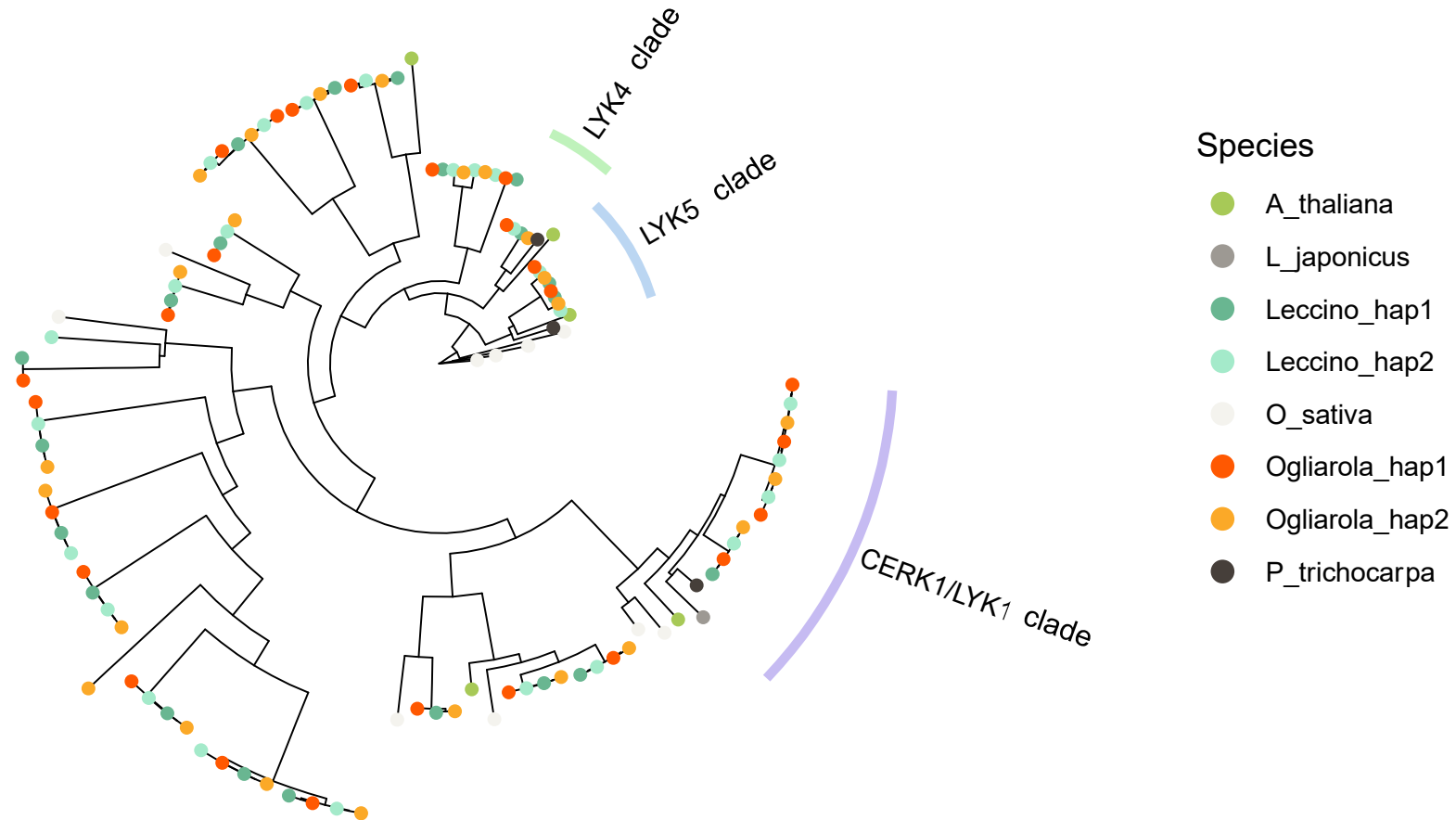
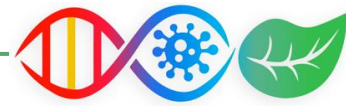
1 = non-immunogenic

2 = weakly immunogenic

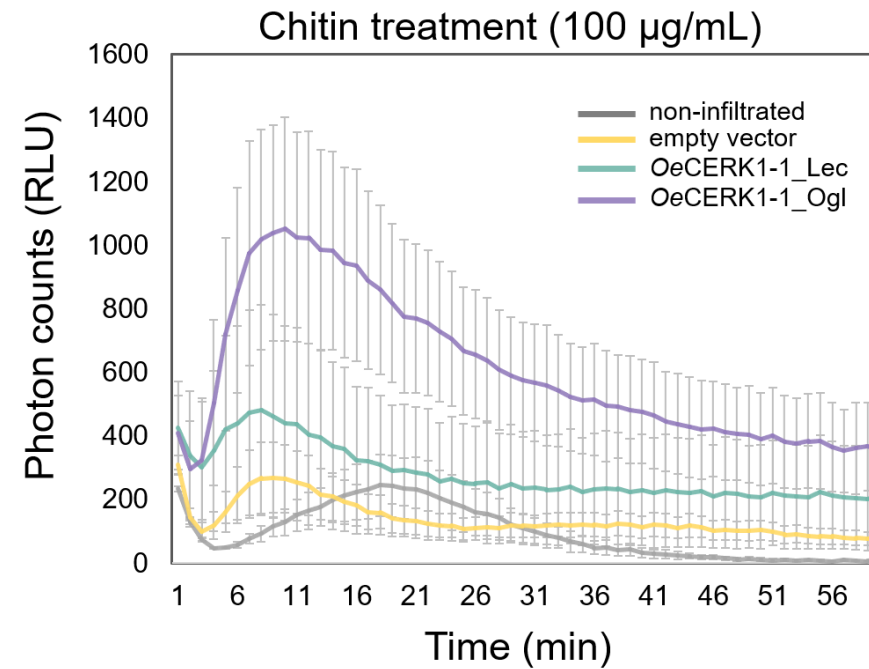
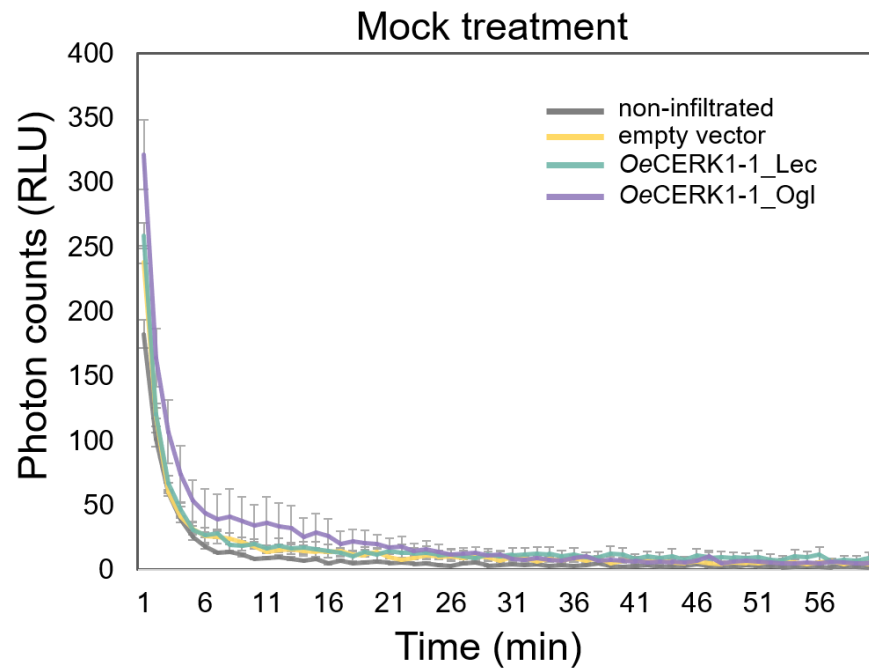
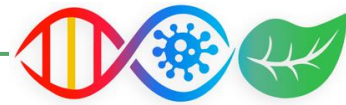
Research focus > MAPK activation induced by microbial patterns and bacterial extracts



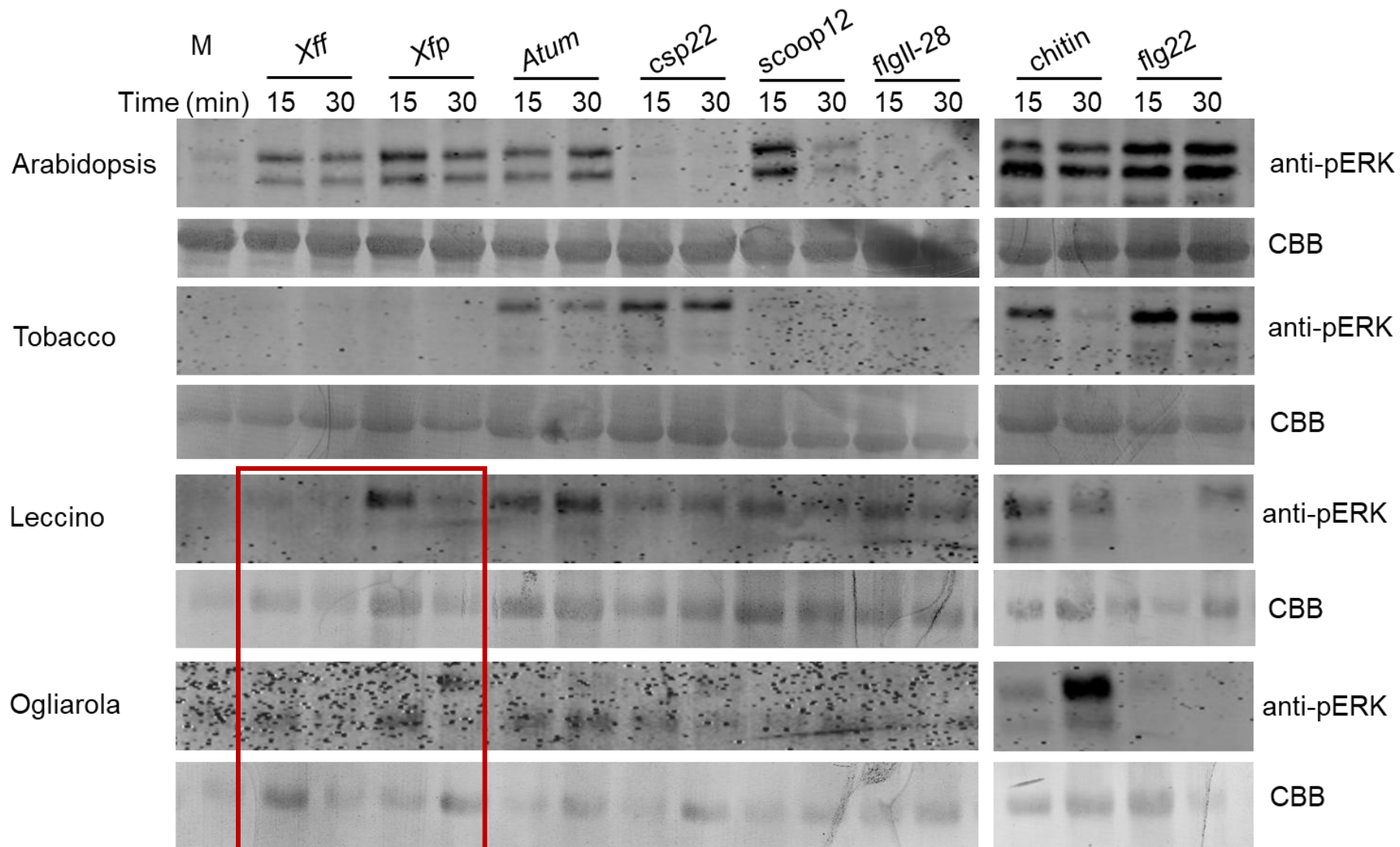
Research focus > Phylogenetic tree of olive LysM-RLKs



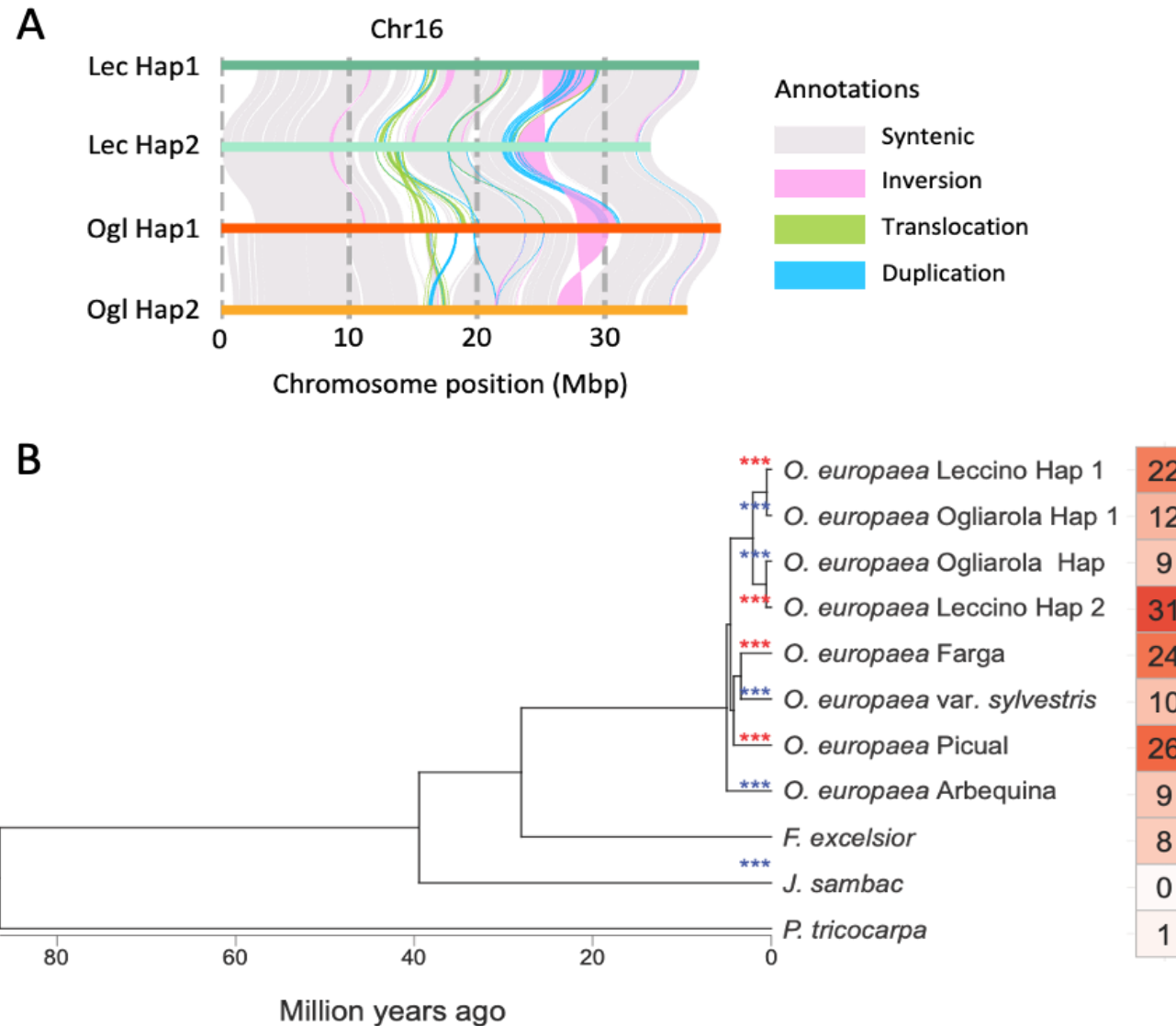
Research focus > Analysis of *OeCERK1-1* from Leccino and Ogliarola salentina



Research focus > MAPK activation induced by microbial patterns and bacterial extracts



Research focus > Identification of an unique region in Leccino chromosome 16



Research focus > Phylogenetic tree of olive RLK-XII



Species

- A_thaliana
- B_rapa
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- S_tuberosum

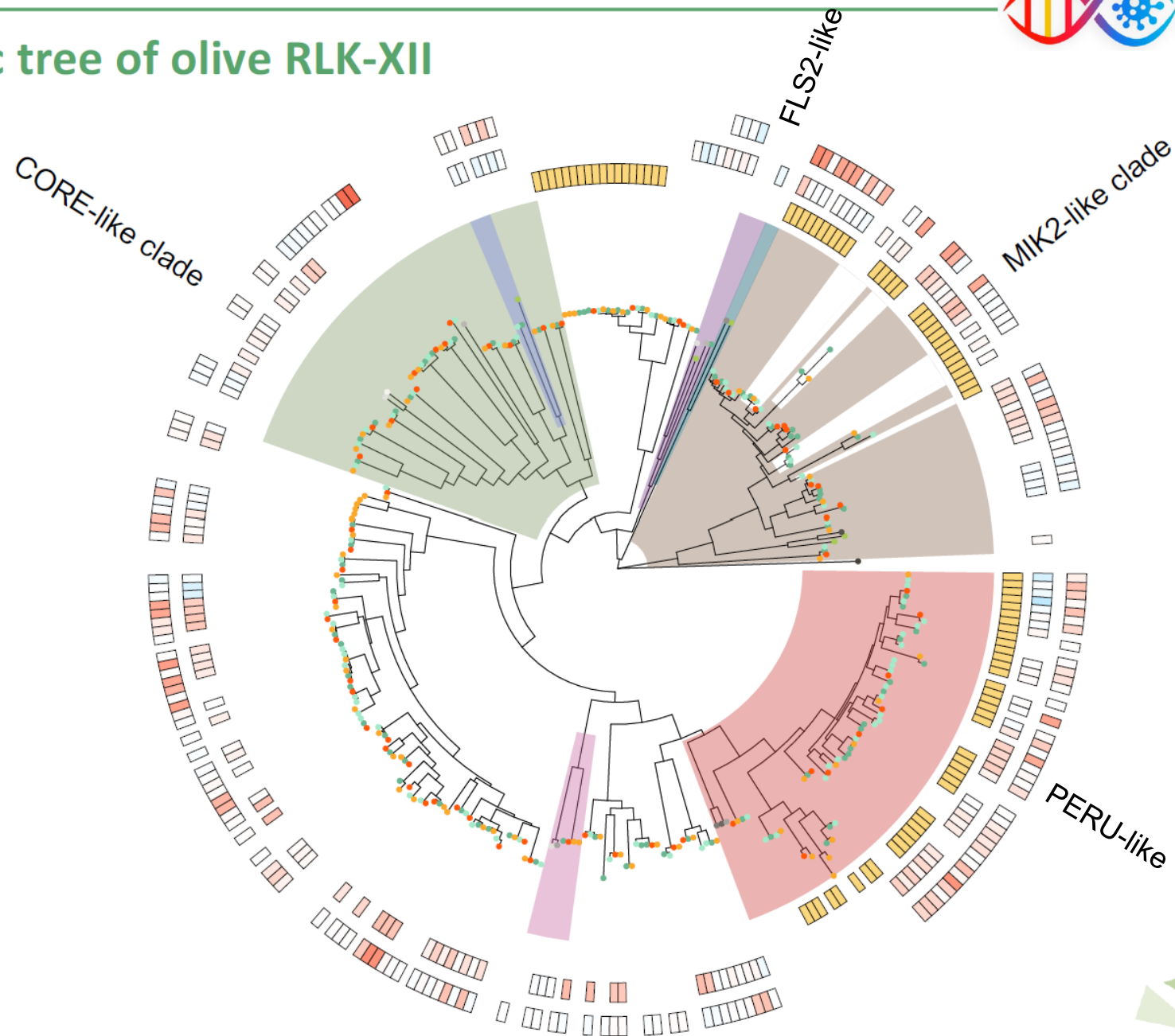
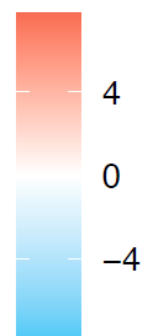
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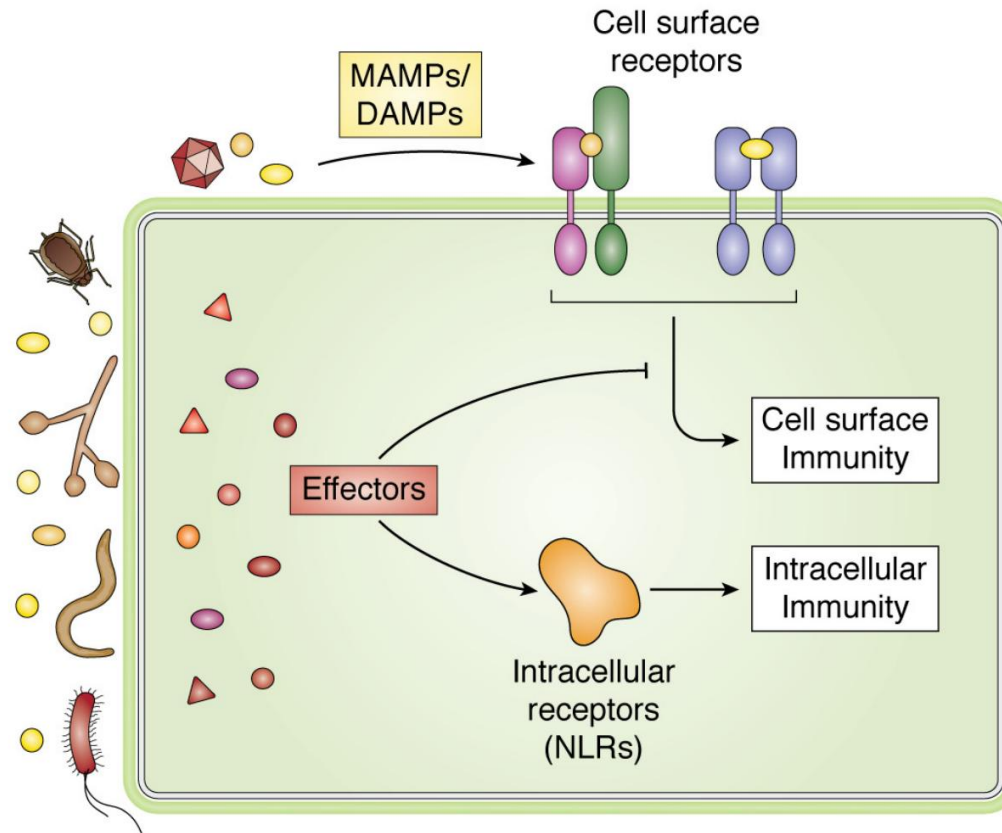
Chr16

■ Chr16

log2FC



> The two tiers of the plant immune system



MAMPs/DAMPs

bacteria: flagellin (flg22, flgII-28)

cold-shock protein (csp22)

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self/non-self: scoop + microbial scoop-like

PRRs

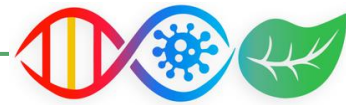
FLS2: no (?)

CORE: ??

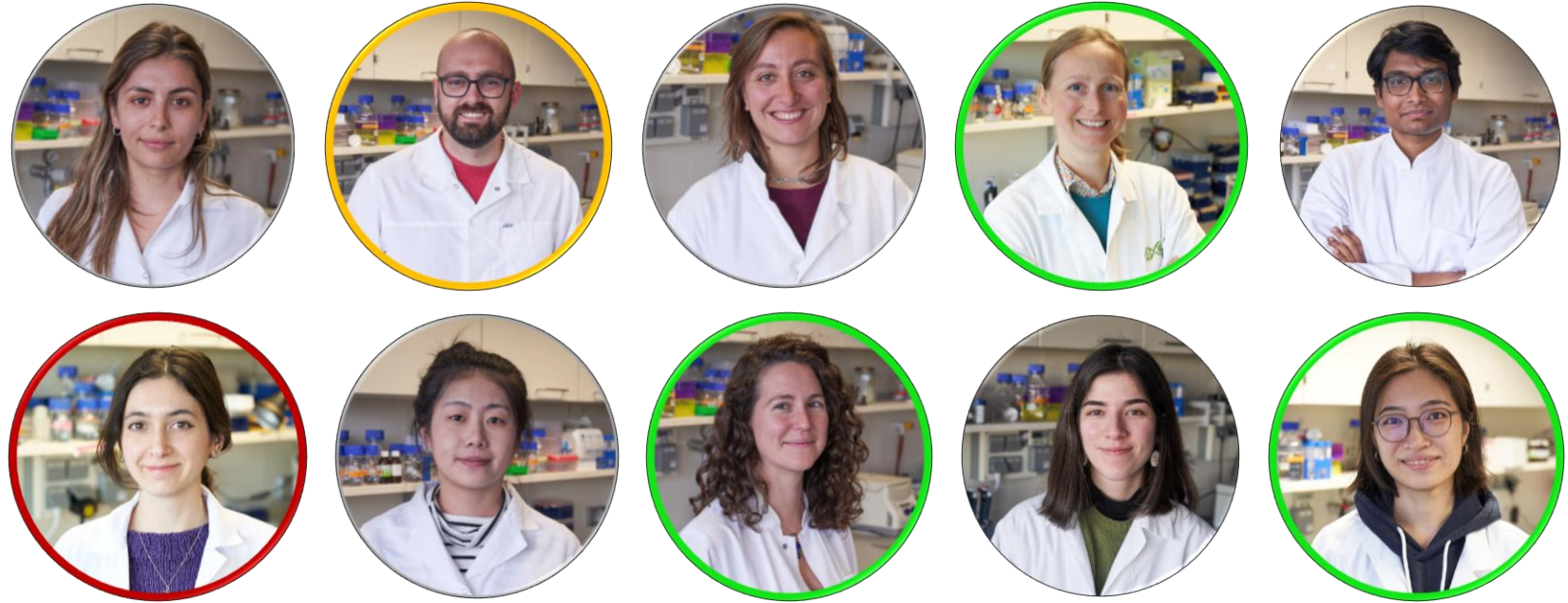
CERK1: yes

MIK2: ??

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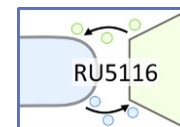


Collaborators

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Blanca Landa (CSIC Cordoba, ES)
Christina Ludwig (BayBioMS, TU Munich)
Pasquale Saldarelli+Maria Saponari (CNR Bari, IT)
Korbinian Schneeberger (LMU Munich)
Ken Shirasu, Yasuhiro Kadota, Bruno Ngou (Riken, JP)
Kai Papenfort (Uni Jena)



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LMU and DFG Equality Funds



Knowledge Transfer Fund



Xylella fastidiosa

5th European conference

Mola di Bari | 23-25.06.2026



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Regeneration of Olive Crop After the Unprecedented Epidemic of *Xylella* *fastidiosa* subsp. *pauca* in Apulia: Key Factors in the Evolving Epidemics

Image: Stefano Petroni

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¹ Centro di Ricerca, Formazione e Sperimentazione in Agricoltura “Basile Caramia” (CRSFA), Locorotondo, Bari Italy;

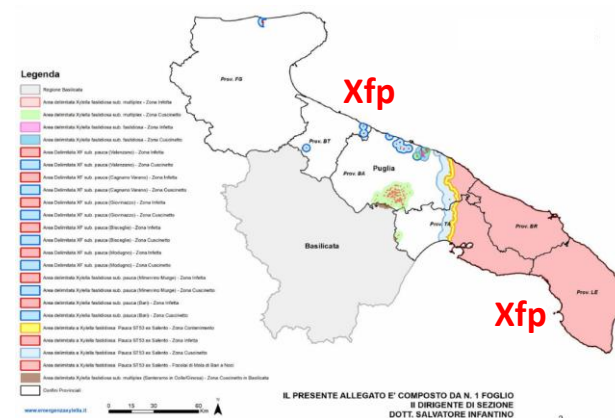
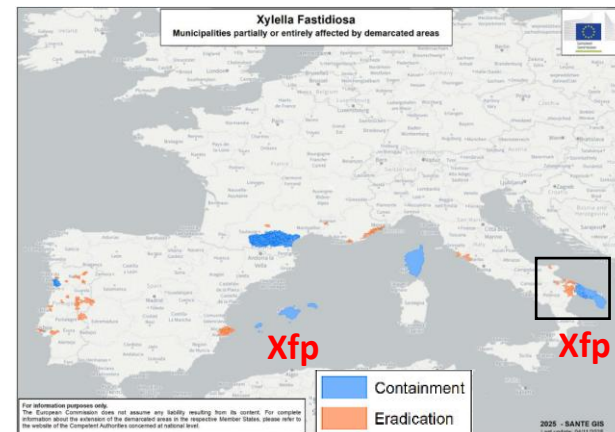
² Università di Brescia, DICATAM, Brescia, Italy;

³ Institute for Sustainable Plant Protection (IPSP), National Research Council, Bari, 70126, Italy

Resistant olive cultivars to contrast *Xf* epidemics

- More than a decade after the first detection, *Xylella fastidiosa* subsp. *pauca* is still a relevant issue in Apulia
- Resistant and tolerant olive cultivars are central to replanting strategies, but resistance does not mean immunity
- Their long-term performance depends on natural inoculum pressure, vector populations and orchard management
- **Objective**

To investigate the **incidence dynamics** of *Xf* infection in newly established olive groves planted with resistant cultivars under different agronomic and vector-density conditions



Experimental design

- Factors considered in the experimental design



Cultivar

- Leccino cultivar (8 groves)
- Fs17 cultivar (8 groves)

16 olive groves

Commercial orchards in the infected area
3-4 years old in 2023



Experimental design

- Factors considered in the experimental design



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- Leccino cultivar (8 groves)
- Fs17 cultivar (8 groves)



Planting density

- Leccino:
 - Low density (130-280 plants/ha)
 - High density (400 plants/ha)
- Fs17:
 - High density (350-400 plants/ha)
 - Super high density (800-1100 plants/ha)

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Soil management

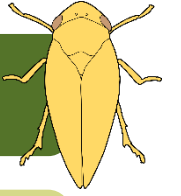
- Soil tillage
- No tillage with mulching

16 olive groves

Commercial orchards in the infected area
3-4 years old in 2023



Vector monitoring



Juvenile and early-adults

- Assessments once a year in late April in 2023-2025
- 30 sampling unit (0.25 m²) in the ground cover vegetation
- Internal and border sampling areas
- *Phileanus spumarius* and *Neophilaenus campestris*



Adult population

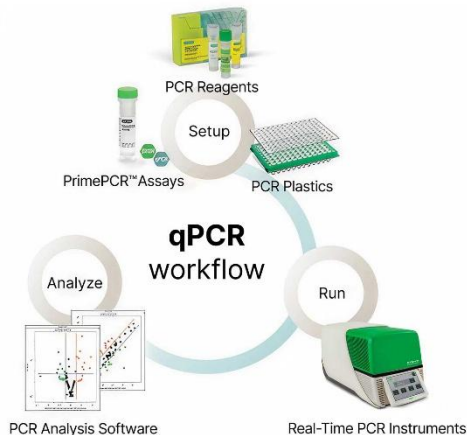
- Once or twice a month from April to October, in 2023-2025
- *Phileanus spumarius* and *Neophilaenus campestris*
- Yellow sticky traps on the olive canopy
 - Internal and external traps
- Sweep nets on the ground cover vegetation
 - Randomly defined within the area



Incidence of infection in olive trees

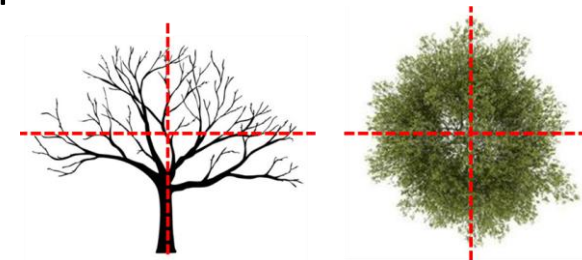
Field level

- Samplings once a year in 2024 and 2025
- 30 to 50% trees sampled in each grove
- 6 shoots per tree
- Detection of Xf by qPCR



Within-canopy level

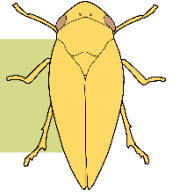
- 3 sampling collections
 - Autumn 2024
 - Spring 2025
 - Autumn 2025
- 6 infected plants selected per grove
- Canopy sampled in 8 sections
 - 4 quadrants in the upper canopy
 - 4 quadrants in the bottom canopy



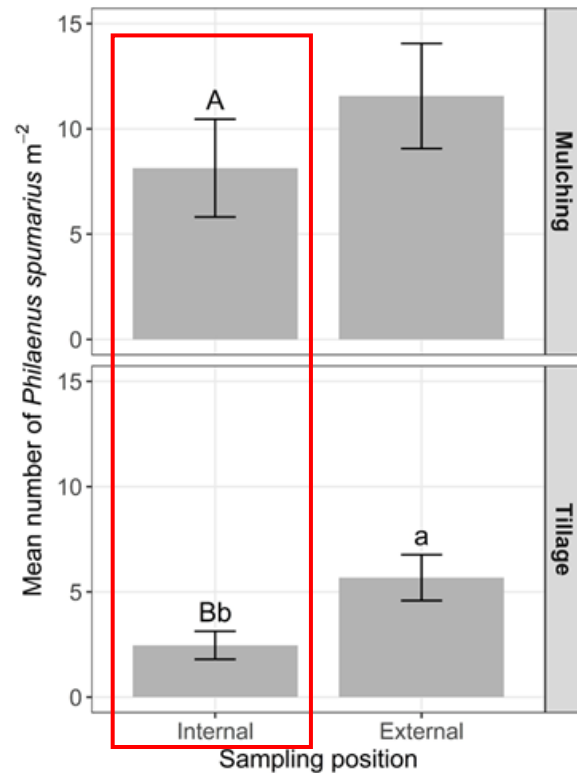
- Detection of Xf by qPCR

Main results

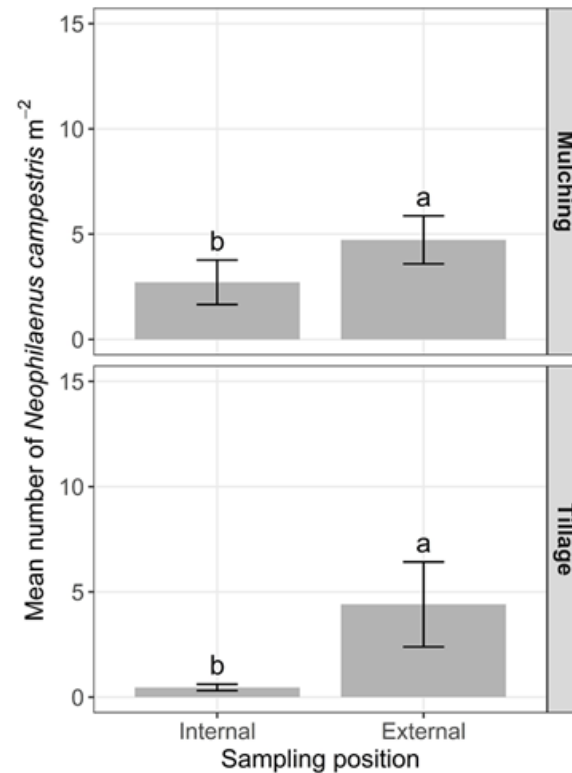
Juvenile and early-adults on the ground cover vegetation



Philaenus spumarius



Neophilaenus campestris

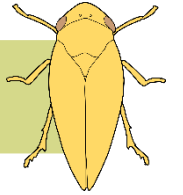


- Higher density of *P. spumarius* than *N. campestris* ($p < 0.001$)
- Significant effect of **soil management** for *P. spumarius* ($p = 0.039$)
 - Overall higher density in **mulching groves**
- Significant effect of sampling position for both *P. spumarius* ($p = 0.007$) and *N. campestris* ($p = 0.001$)
 - Overall higher along field **borders/external areas**

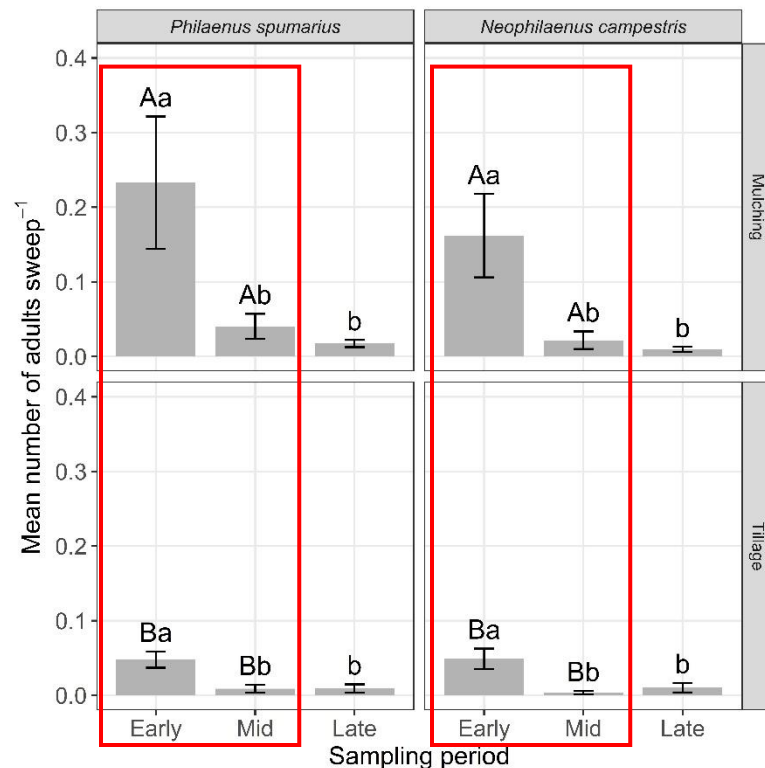
Different lowercase letters differ significantly among sampling positions
Different uppercase letters between soil-management

Main results

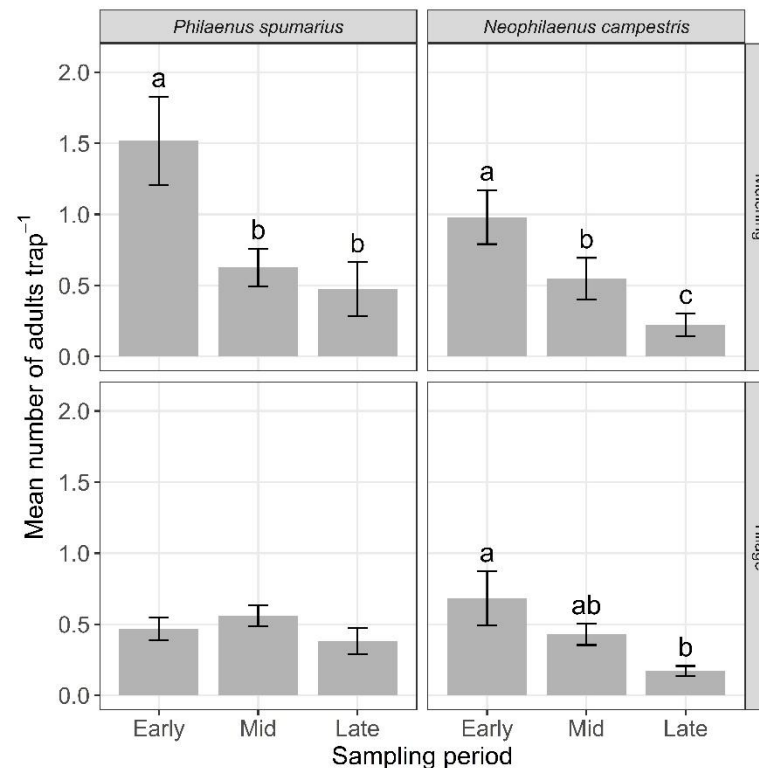
Adult population



Sweep-net on the ground cover vegetation



Yellow sticky traps (canopy)

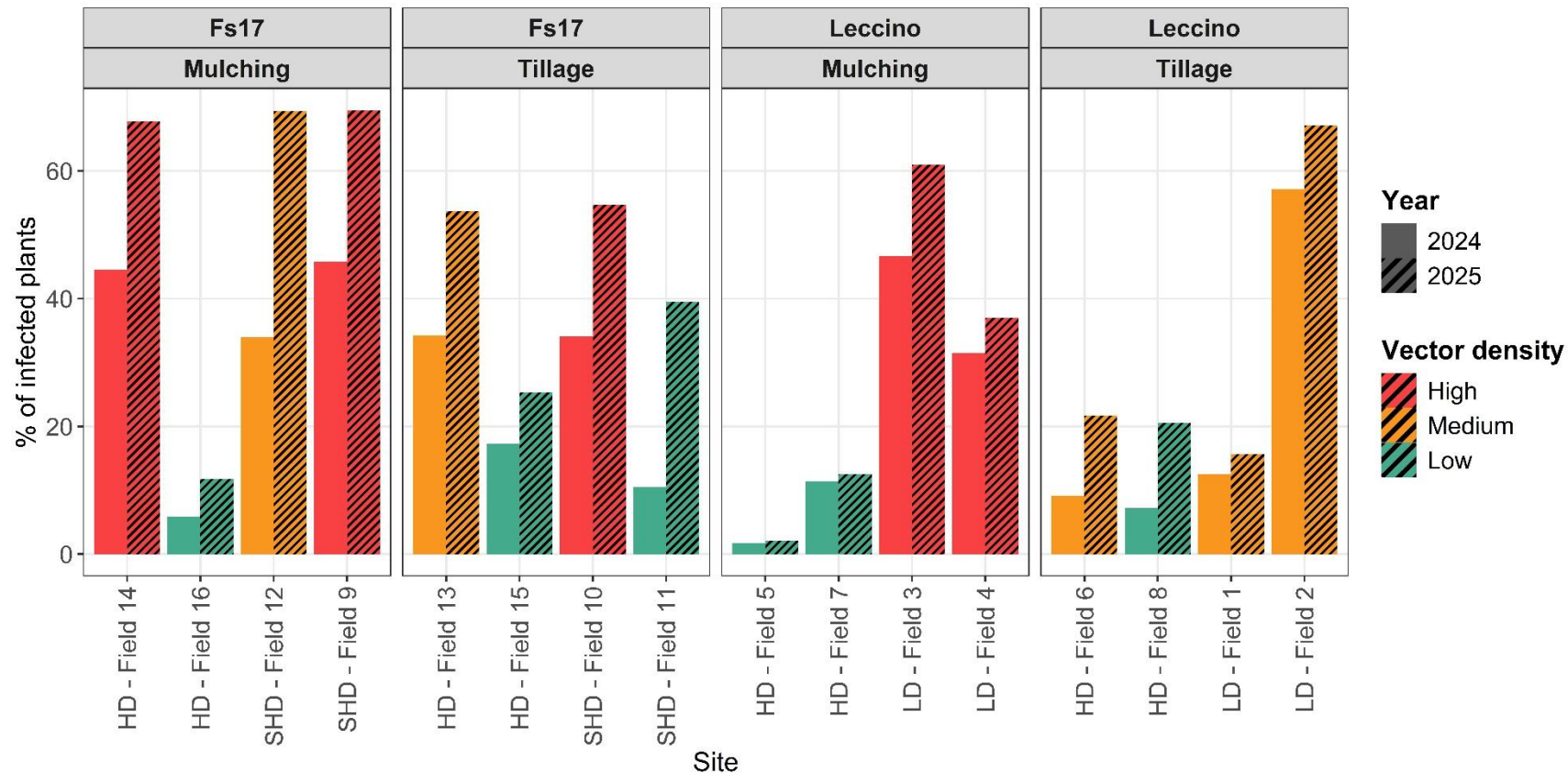


- No significant differences between *P. spumarius* and *N. campestris* ($p = 0.231$ in traps and 0.390 in sweep-nets)
- Significant effect of **soil management** for insect density on the ground cover vegetation ($p = 0.005$)
 - Overall higher density in **mulching groves**
- No significant effect of **sampling position** for both *P. spumarius* ($p = 0.519$) and *N. campestris* ($p = 0.614$)

Different lowercase letters differ significantly among sampling periods
Different uppercase letters between soil-management

Main results

Incidence of Xf infection in olive trees



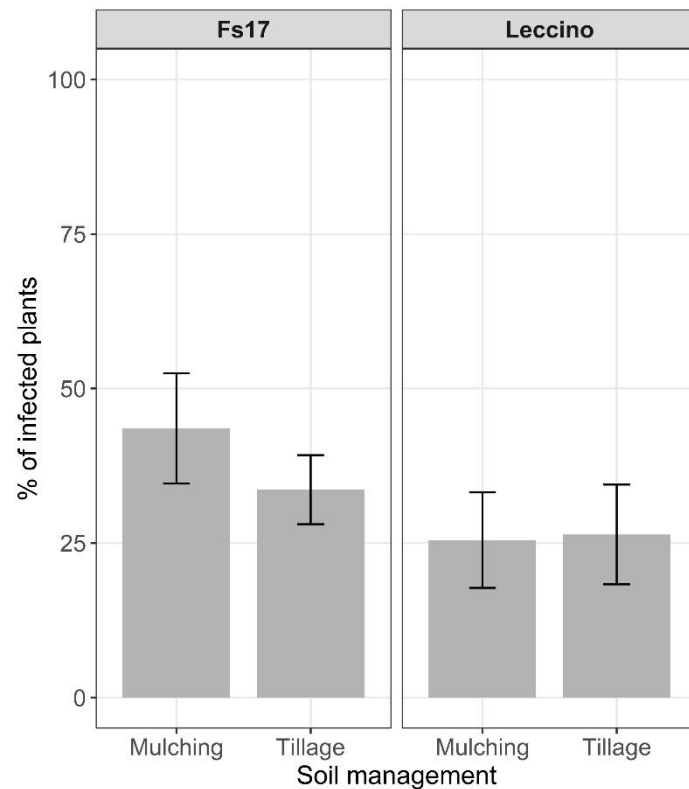
- Increase in the proportion of infected trees over time in all fields
- **Absent or very limited symptoms** of Xf infection observed in infected trees even in fields with high incidence of infection

Main results

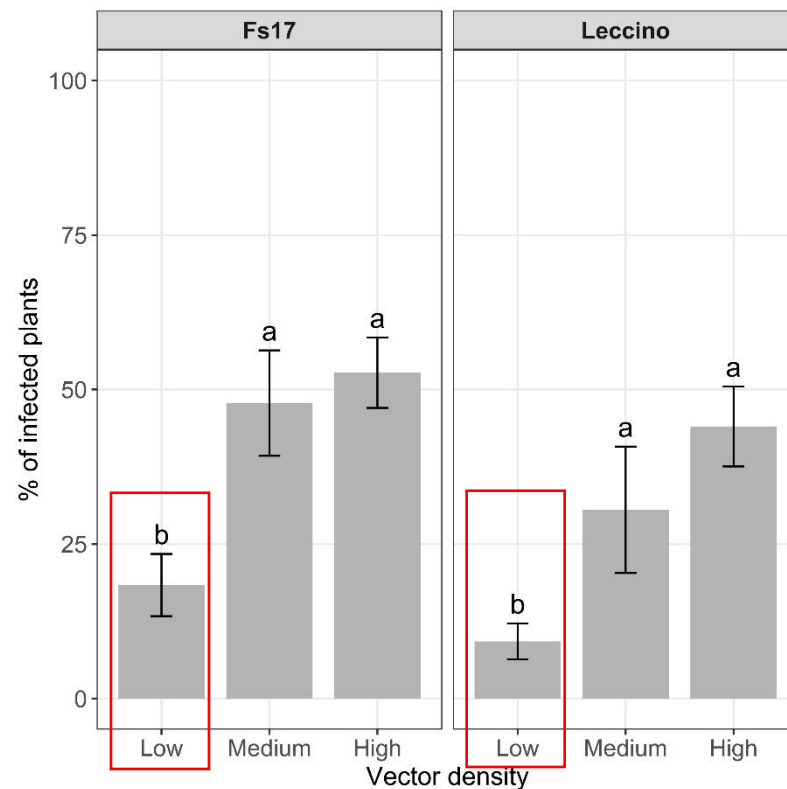
Incidence of Xf infection in olive trees



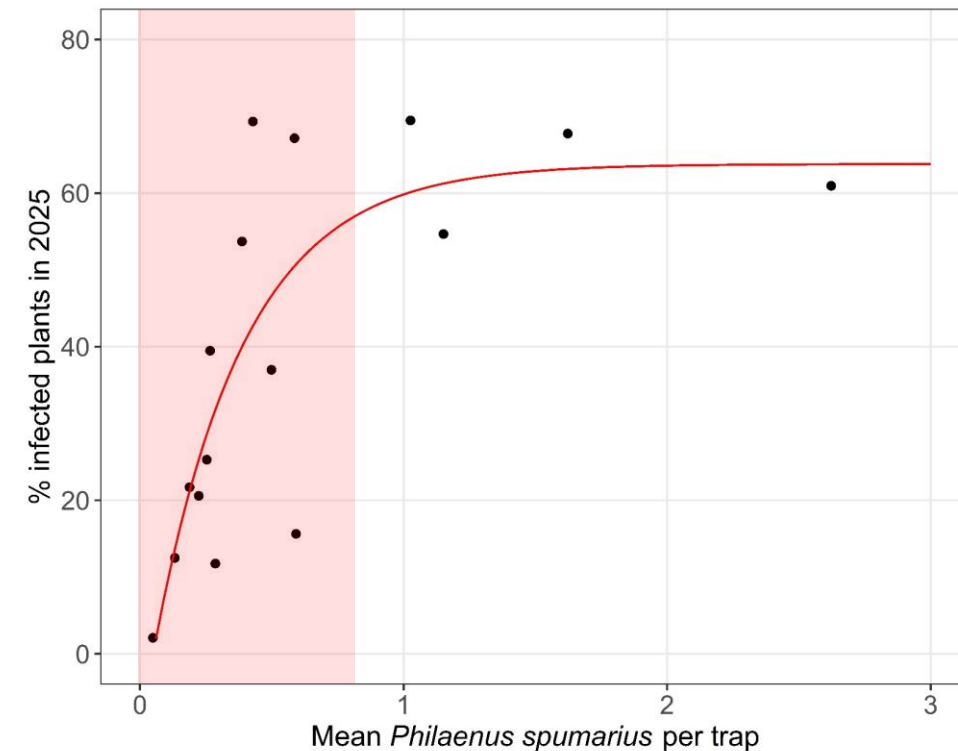
No detectable effect of **soil management** ($p = 0.961$)



Significant effect associated with **cultivar** ($p = 0.023$) and **vector density** ($p < 0.001$)



Correlation between **incidence of infection** and ***P. spumarius* density** (Pearson's $r = 0.61$, $p = 0.013$)



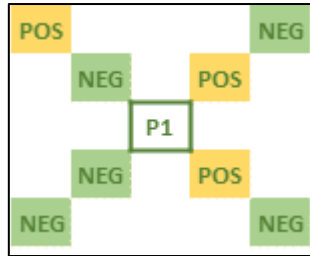
Different lowercase letters differ significantly among vector density

Main results

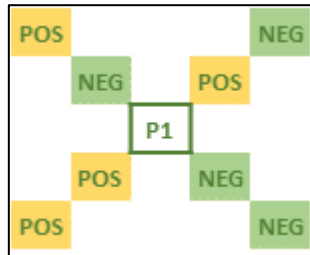
Within-canopy infection dynamics

Heterogeneous and dynamic within canopy detection

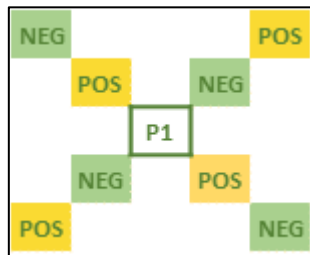
Autumn 2024



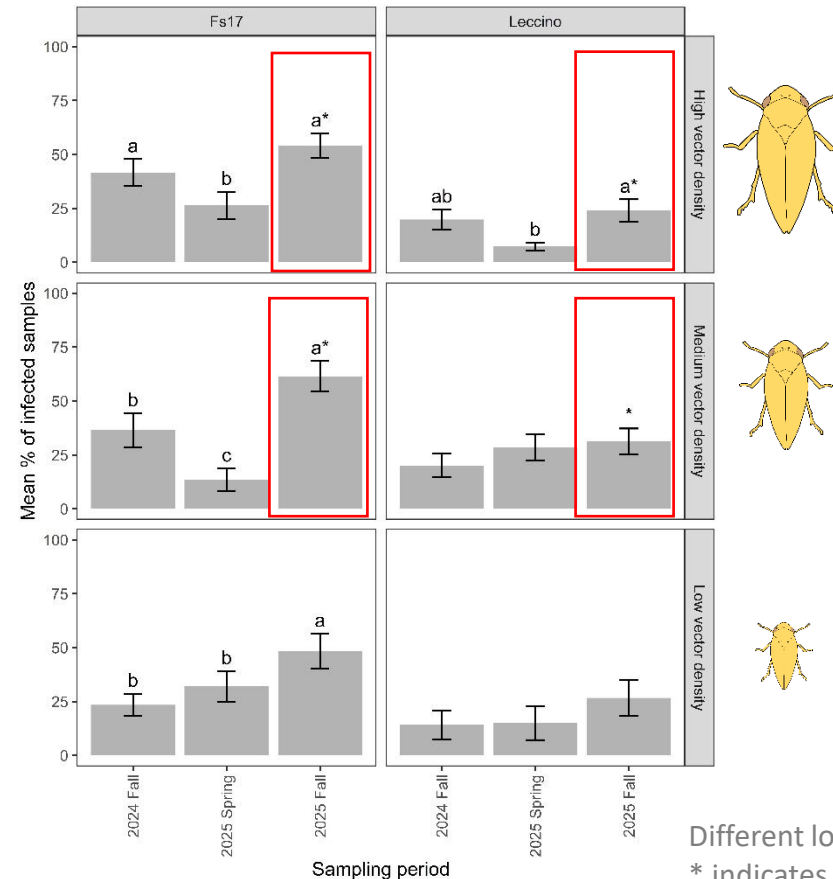
Spring 2025



Autumn 2025



Cultivar x Sampling period x Vector density



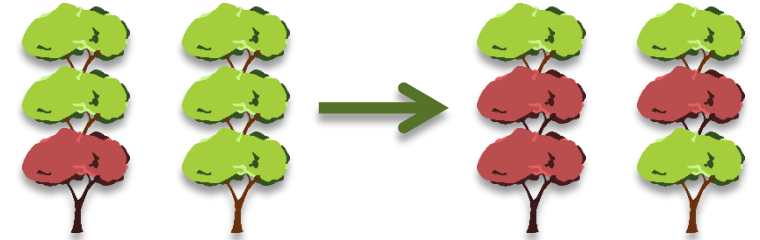
- Significant interaction between cultivar × sampling period × vector-density ($p = 0.0057$)
- **Reduction** in the proportion of infected branches in **spring 2025** in some cases
- No vector-density class differed within any sampling period

Different lowercase letters differ significantly among sampling periods
* indicates significant differences between cultivars

Take-home messages

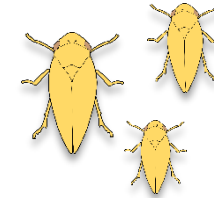
Infection continued

Both resistant cultivars became infected, and incidence increased in all monitored groves



Vector pressure mattered

Medium- and high-density fields were associated with substantially greater infection burden than low-density fields



Soil management effects were mixed

Tillage reduced vectors on ground cover, but no field-level difference in infection incidence was detected



Regeneration requires integrated management

Resistant cultivars support the regeneration of olive production, but sustained vector suppression and phytosanitary measures remain necessary to slow evolving epidemics



**Università
di Brescia**
AgroFood Research Hub



Funded by the
European Union

A large, ancient-looking tree with a thick, gnarled trunk and dense green foliage, set against a clear blue sky with some light clouds. The tree is the central focus of the background image.

Thank you!

Image: Stefano Petroni

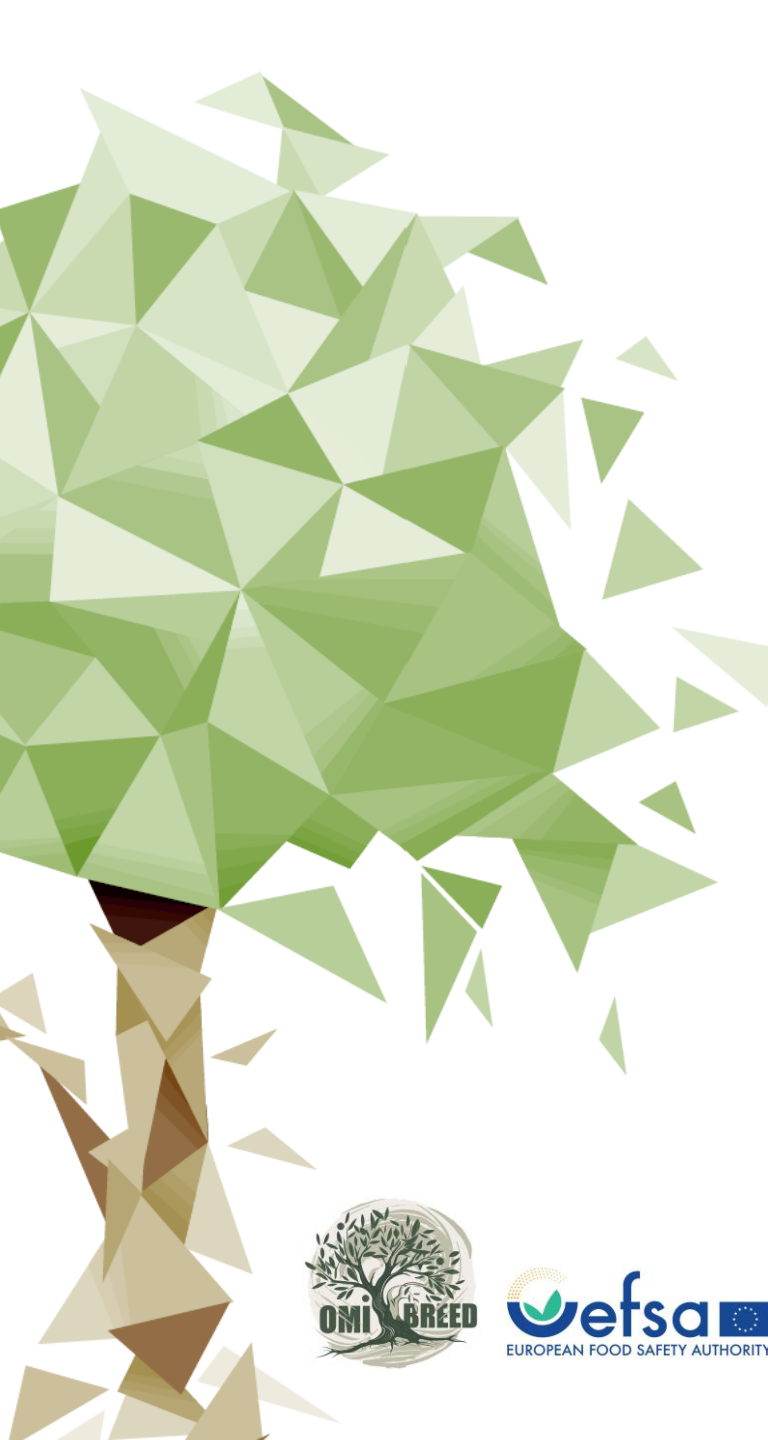
The BeXyl WP7 team

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Università di Brescia





Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026

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

Giampetruzzi A., de Souza-Neto R. R., Boscia D., Saldarelli P., Spanò R.,
Abou Kubaa R., Saponari M.





190 RNA-Seq libraries



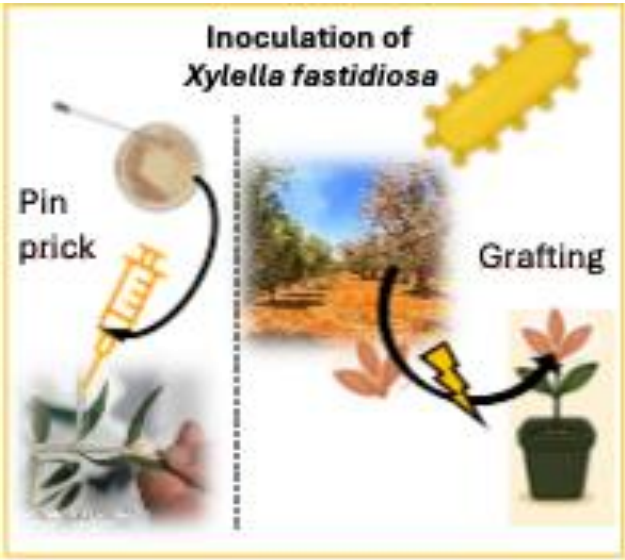
Early

Chronic



Cellina
Ogliarola
Leccino 40
FS17
Frantoio
s105, s215(LecXCipr)
s234(LecXOgl)
S218(LecXCel)
Arbequina
Leccino
Lecciana

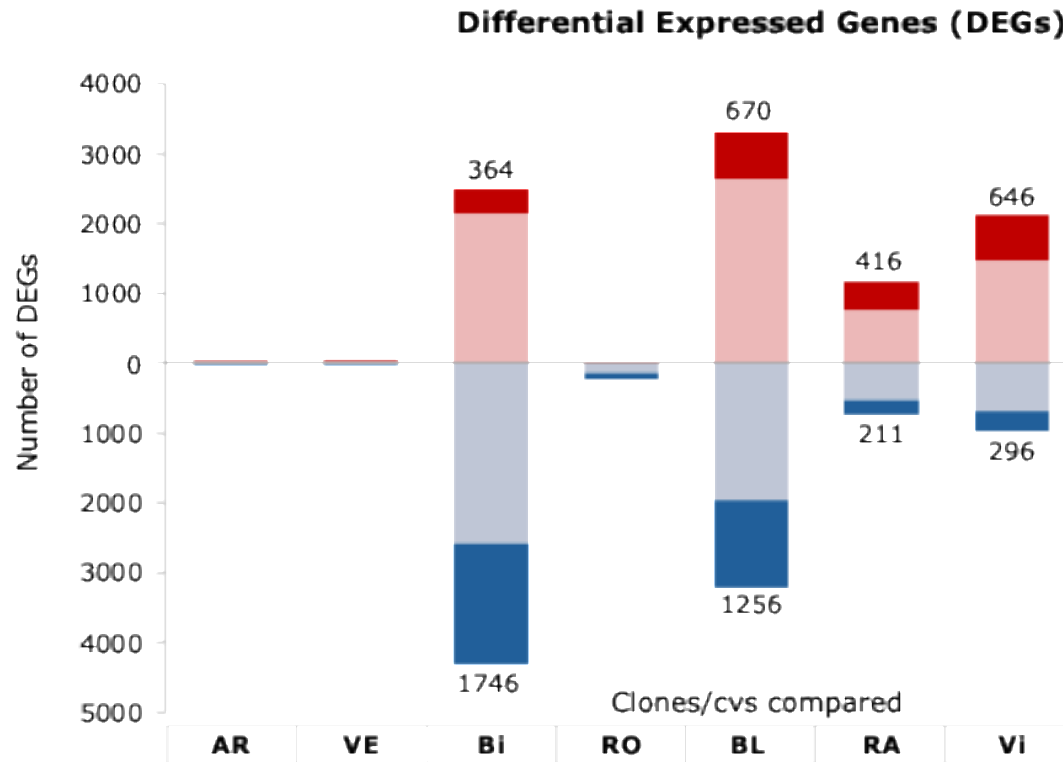
4



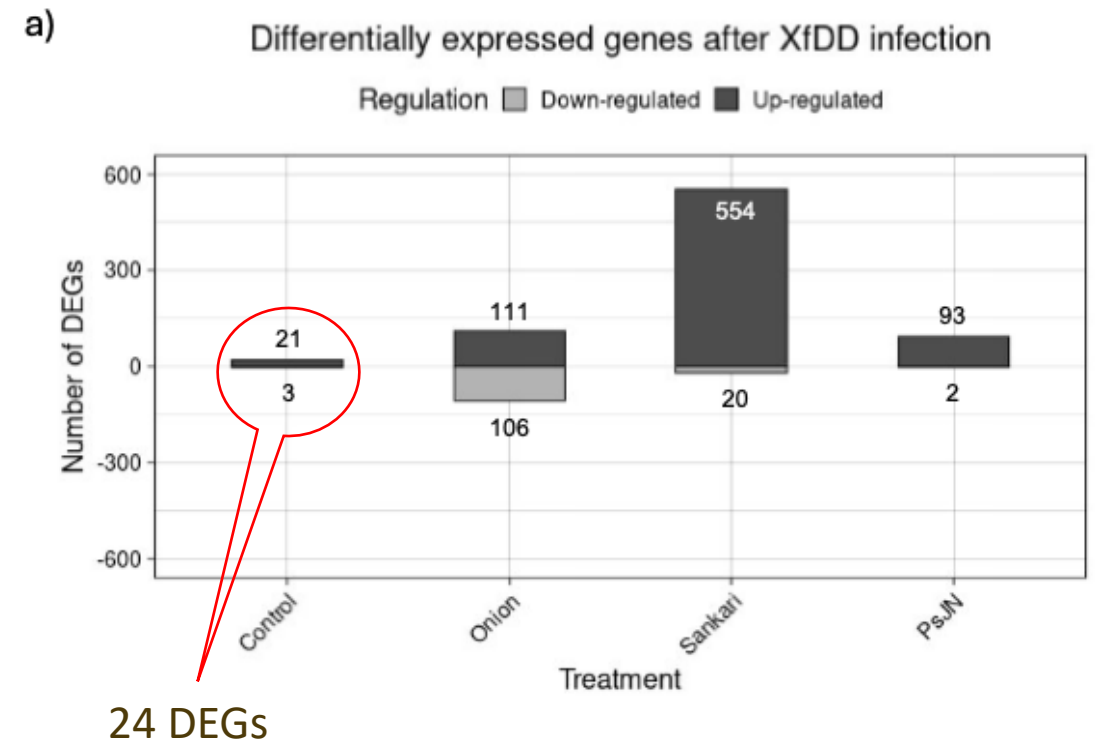
Cellina	25
Ogliarola	3
Leccino	53
FS17	5
Frantoio	8
S105,s215	10
LecXOgl	5



Does olive sense Xylella? How early?



48hpi Abou Kubaa et al 2026
Leccino



10dpi Giampetruzzi et al 2026
Ogliarola s.

CONSERVED TRANSCRIPTIONAL RESPONSES

chronic

Are there recurrent pathways/DEGs among $\neq$ RNASeq analysis?



Leccino



S234 (Leccino X Ogliarola s.)



S105 (Leccino X Cipressino)



Cellina



Xfp-infected
plants **16**
months
(16mvpi)

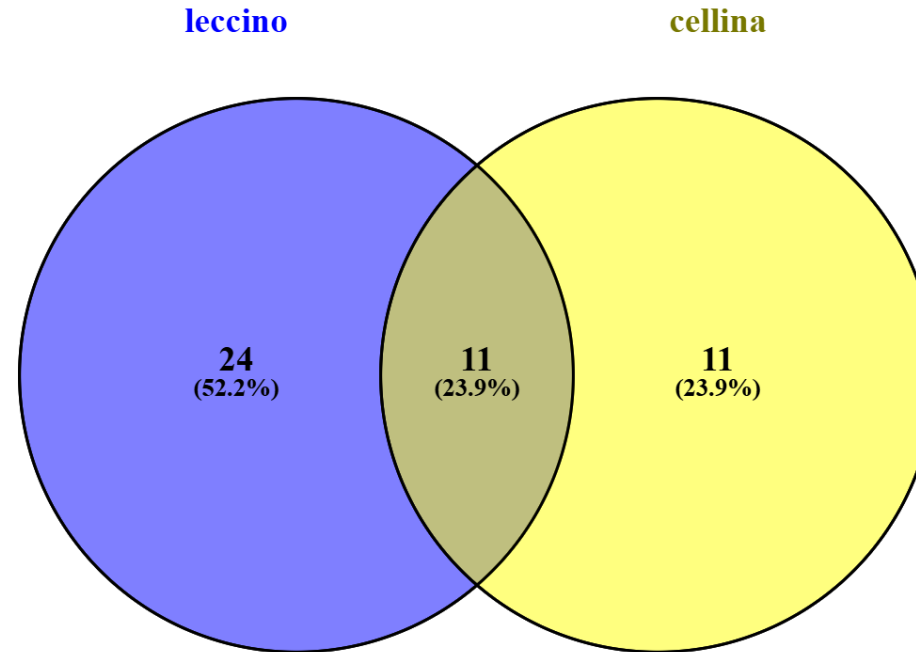


Xfp-infected
plants **24**
months
(24mvpi)

CONSERVED TRANSCRIPTIONAL RESPONSES

Are there recurrent pathways/DEGs among ≠ RNASeq analysis?

Pathways enriched by DEGs



24 elements included exclusively in Leccino

GO:0009832 Plant-type cell wall biogenesis

GO:0042546 Cell wall biogenesis

GO:0016705 Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen

11 elements included exclusively in Cellina:

GO:0006771 Riboflavin metabolic process

GO:0005623 Cell

GO:0003993 Acid phosphatase activity

GO:0048544 Recognition of pollen

GO:0043565 Sequence-specific DNA binding

GO:0006826 Iron ion transport

GO:0016311 Dephosphorylation

GO:0003700 Transcription factor activity, sequence-specific

DNA binding

GO:0015994 Chlorophyll metabolic process

GO:0006879 Cellular iron ion homeostasis

GO:0004322 Ferroxidase activity

11 common elements in "leccino" and "cellina":

GO:0006468 Protein phosphorylation

GO:0004672 Protein kinase activity

GO:0055114 Oxidation-reduction process

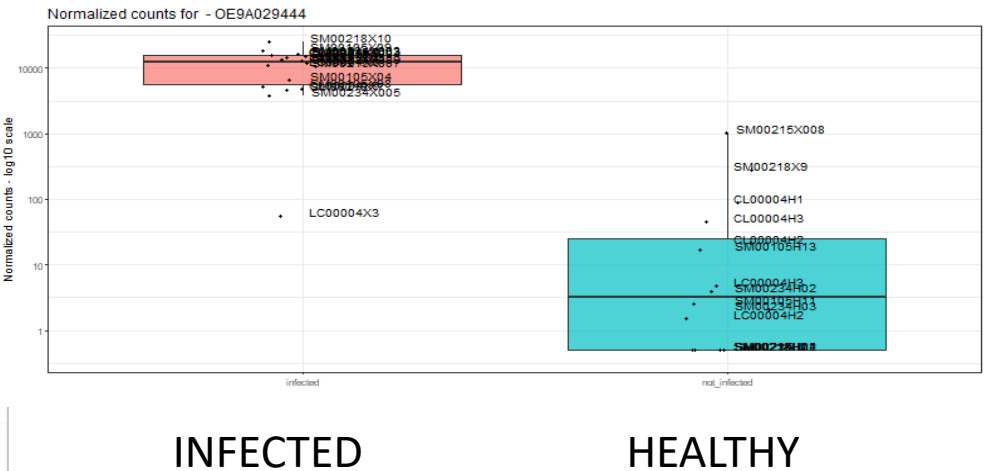
GO:0009607 Response to biotic stimulus

GO:0006952 Defense response

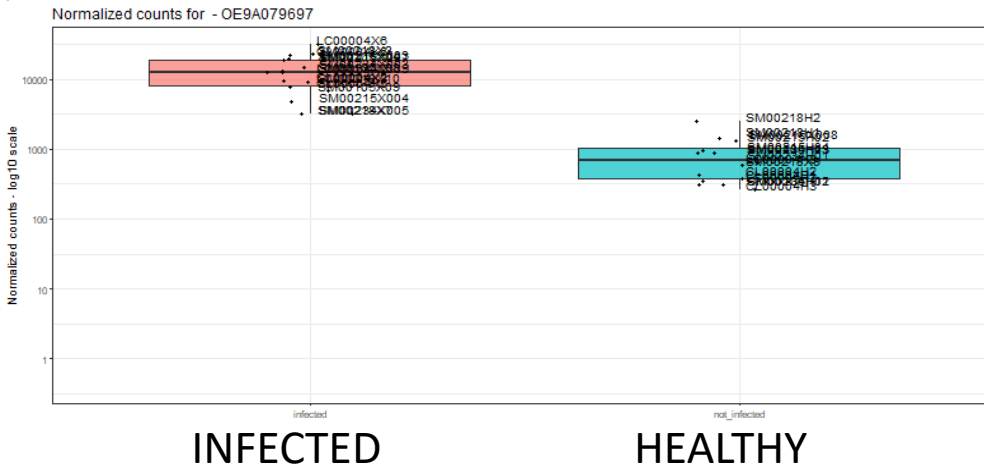
CONSERVED TRANSCRIPTIONAL RESPONSES

Are there recurrent pathways/DEGs among ≠ RNASeq analysis?

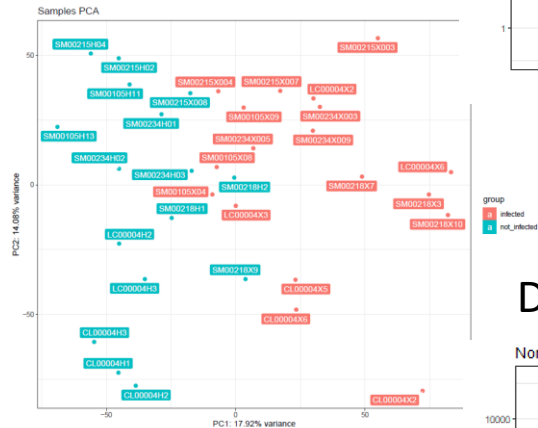
Putative EG45-like domain containing protein 1



Description=DOWNY MILDEW RESISTANCE 6-like



1	OE9A029444	Putative EG45-like domain containing protein 1
2	OE9A117543	pathogenesis-related leaf 6-like
3	OE9A094670	Thaumatin
4	OE9A086946	pathogenesis-related STH-2-like
5	OE9A058974	Putative EG45-like domain containing protein
6	OE9A013998	probable LRR receptor-like serine threonine-kinase At3g47570
7	OE9A079697	DMR6
8	OE9A053389	probable nucleoredoxin 1
9	OE9A116998	probable WRKY transcription factor 70



CONSERVED TRANSCRIPTIONAL RESPONSES

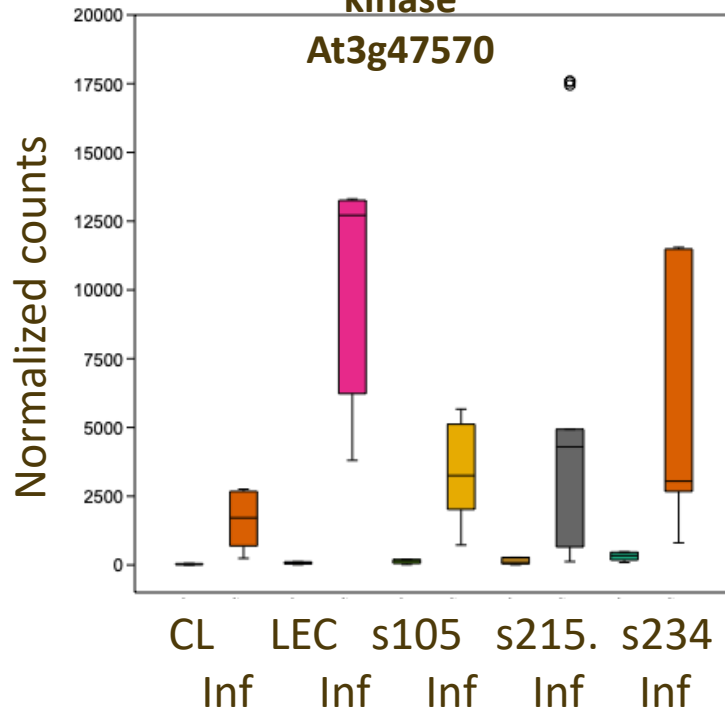
Are there recurrent pathways/DEGs among ≠ RNASeq analysis?

Leccino log2fold change 6-5.4
Cellina log2fold change 4.6-3.3

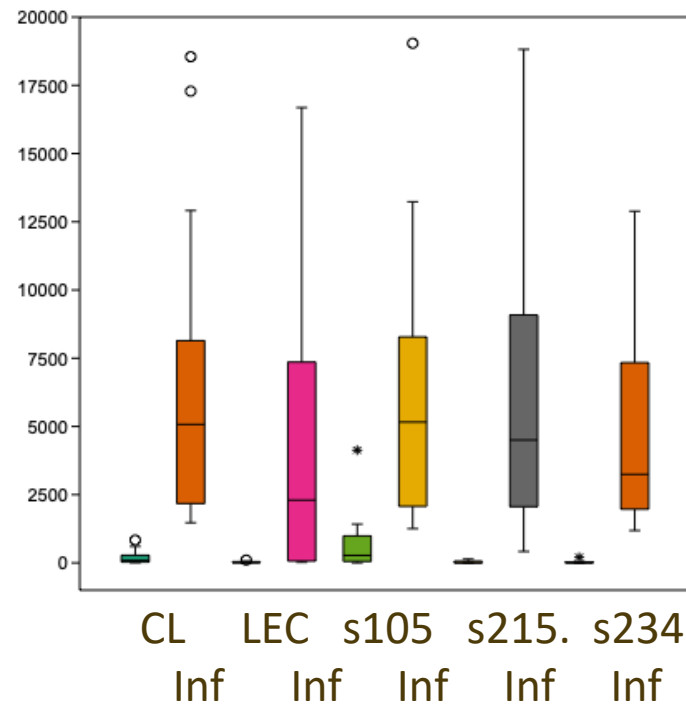
Leccino log2fold change 9.5-5
Cellina log2fold change 4.6-2.8

Leccino log2fold change 4.3- 3.2
Cellina log2fold change 1.9-1.7

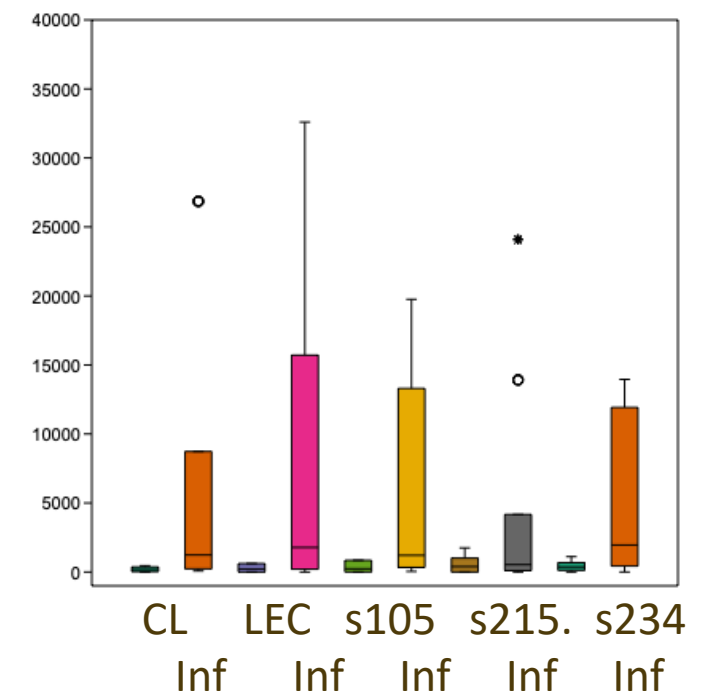
Probable LRR receptor-like serine threonine kinase



Putative EG45-like domain containing protein



DOWNY MILDEW RESISTANCE 6-like and DMR6



All pairs significant

CONSERVED TRANSCRIPTIONAL RESPONSES

Are there recurrent pathways/DEGs among $\neq$ RNASeq analysis?

Principal Component Scores

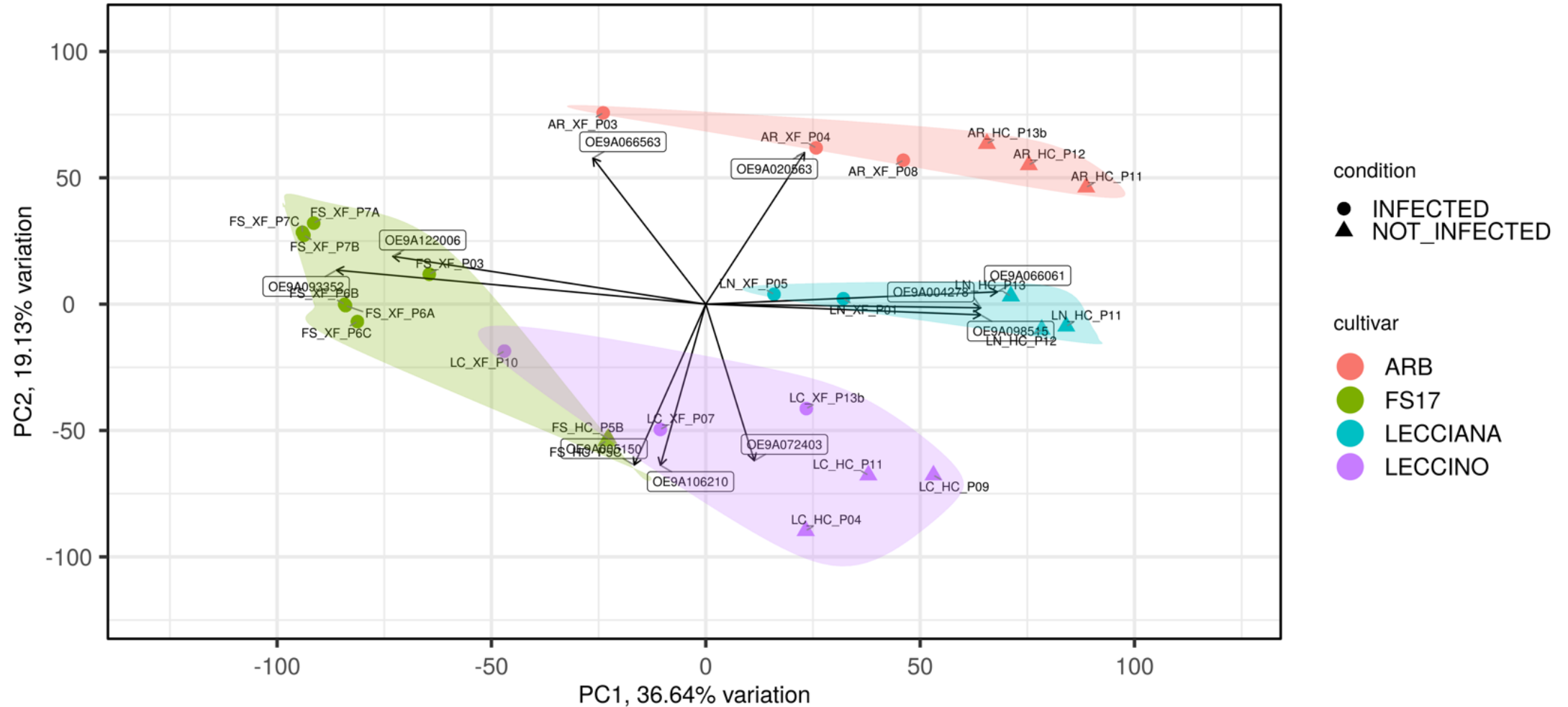


FOTO piante?????

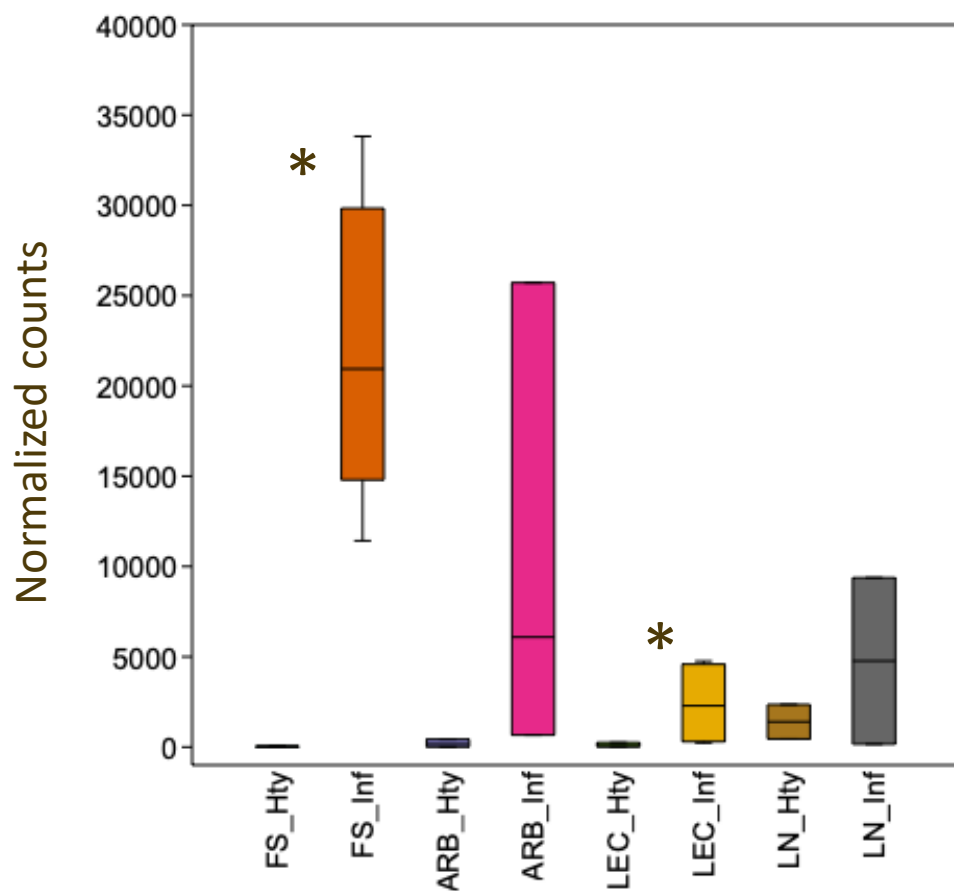
CONSERVED TRANSCRIPTIONAL RESPONSES

Are there recurrent pathways/DEGs among $\neq$ RNASeq analysis?

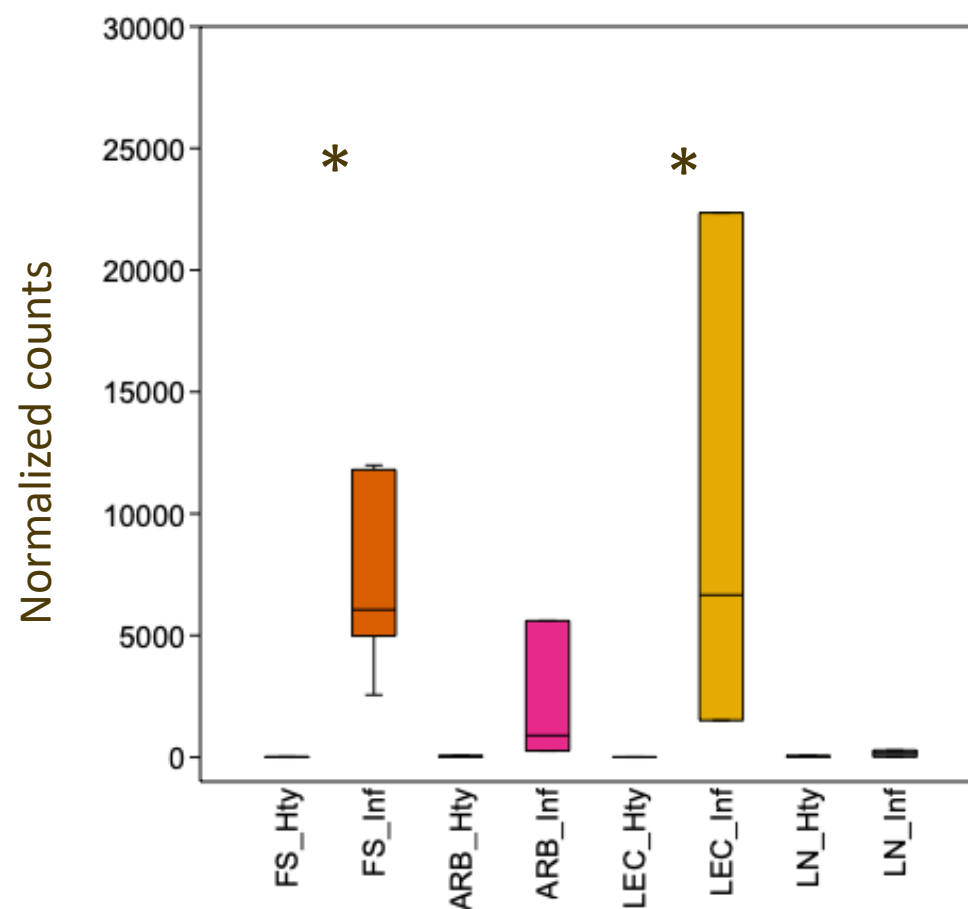
FS log2 fold change 11.4/8.8
ARB log2 fold change 8.4/5.5
LEC log2 fold change 12.9

FS log2 fold change 9.8/3.9
ARB log2 fold change 5.8/2.4
LEC log2 fold change 10.7/4.7/3.4

EG45-like domain containing protein

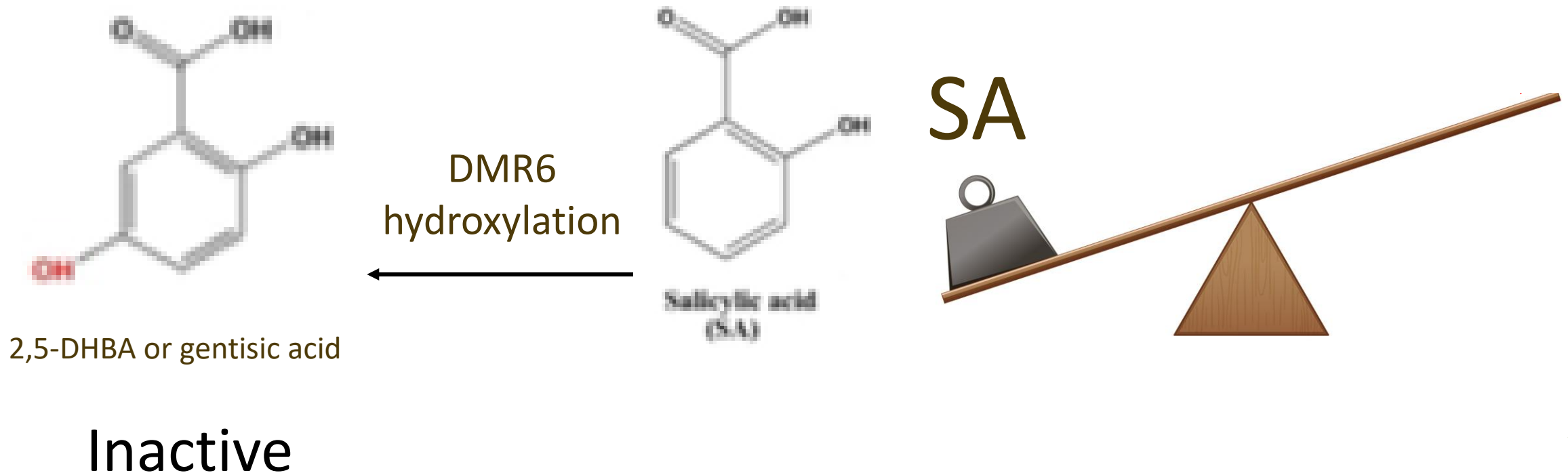


DOWNY MILDEW RESISTANCE 6-like and DMR6



CONSERVED TRANSCRIPTIONAL RESPONSES
Are there recurrent pathways/DEGs among ≠ RNASeq analysis?

NEED TO CONTROL salicylic acid (SA) LEVELS



CONSERVED TRANSCRIPTIONAL RESPONSES

Are there recurrent pathways/DEGs among $\neq$ RNASeq analysis?

likely a salicylic acid (SA)

immune response



reduced growth

leaf yellowing

ROS production (toxicity, cell death)

secondary metabolites (antimicrobial, lignin)

SA



growth – defense trade-off



ENERGY (C and N)

NEED TO CONTROL SA LEVELS

CONSERVED TRANSCRIPTIONAL RESPONSES

Are there recurrent pathways/DEGs among ≠ RNASeq analysis?

NEED TO CONTROL SA LEVELS

EG45-like domain containing protein is homologous to Plant natriuretic peptide A (PNP-A)

PNP-A
antagonizes SA
in *Arabidopsis t.*

SA



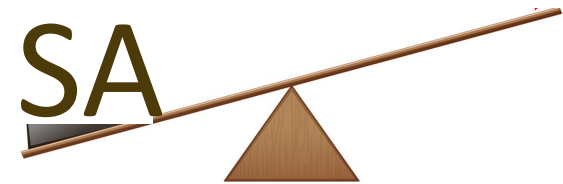
CONSERVED TRANSCRIPTIONAL RESPONSES

Are there recurrent pathways/DEGs among ≠ RNASeq analysis?

NEED TO CONTROL SA LEVELS

PNP-A (EG45) antagonizes SA

PNP-A (EG45) interacts with receptor-like protein, **PNP-R2** in *Arabidopsis t.*



PNP-R2 distantly homologous to several olive RNASeq recurring LRR-RLKs

- LRR receptor-like serine threonine-kinase GS01
 - probable LRR receptor kinase At3g35710
- probable LRR receptor-like serine threonine-kinase At3g47570

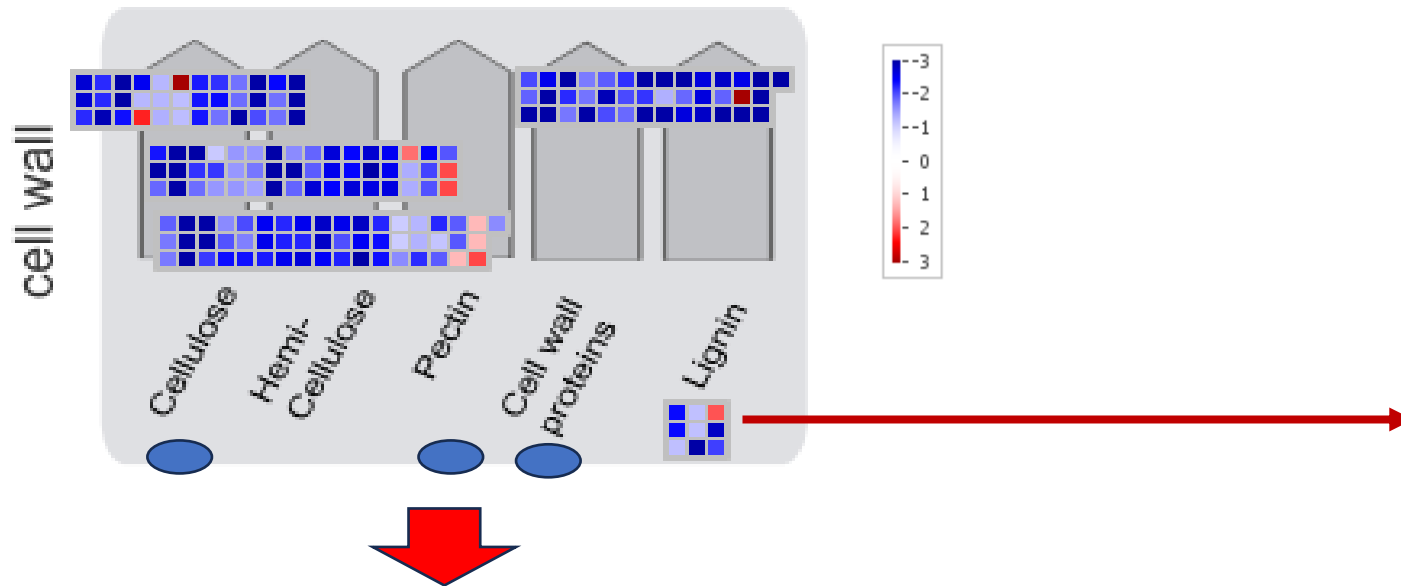
SA

A wooden seesaw is shown, tilted upwards on a triangular fulcrum. A black square weight is placed on the left end of the seesaw. The seesaw is made of light brown wood, and the fulcrum is a darker brown triangle. The background is white.

	log2FoldChange			
OLEA9_A083386	2,941660711	Beta-glucosidase-like		
OLEA9_A113410	2,631312173	Expansin-like EG45 domain-containing protein		
OLEA9_A029444	2,553058923	Expansin-like EG45 domain-containing protein		
OLEA9_A070907	2,392800154	EG45-like domain containing proteins		
OLEA9_A058974	2,329388683	EG45-like domain containing proteins		
OLEA9_A094670	2,232704223	Thaumatococcus-like protein		
OLEA9_A014603	1,60578856	Pathogenesis-related R major form		
OLEA9_A044804	1,544290658	Pathogenesis-related R major form		
OLEA9_A025476	1,447724427	Basic endochitinase-like		
OLEA9_A057882	1,429225294	Beta-glucosidase-like		
OLEA9_A100691	1,250318318	Pathogenesis-related protein 5		

WHAT (likely) MAKES LECCINO RESISTANT?

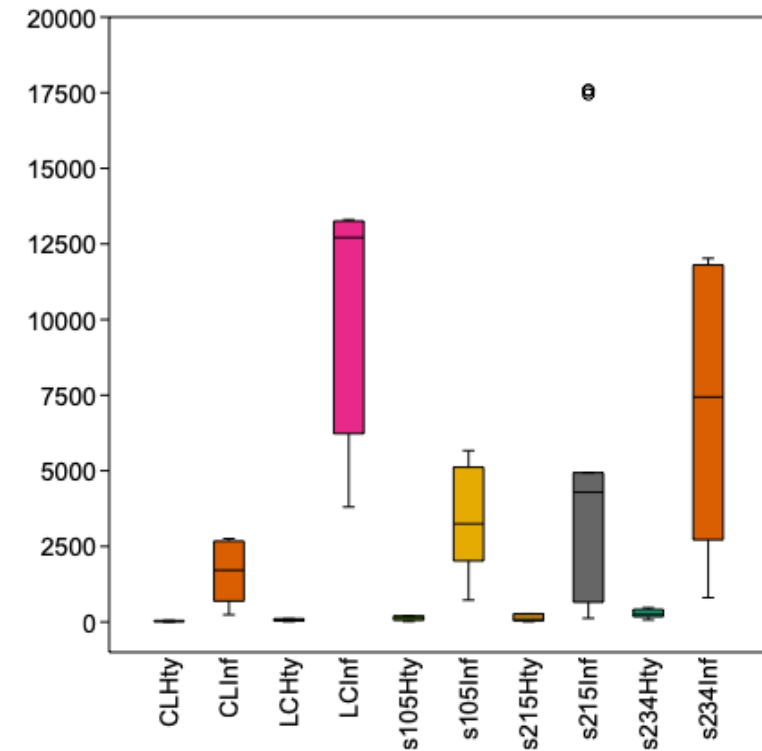
DOWNREGULATION OF CELL WALL GENES IN LECCINO



- Diversion from synthesis to defense (energy trade-off)
- SA induces Lignin deposition in xylem

different Multicopper oxidases (laccases) log2 fold change

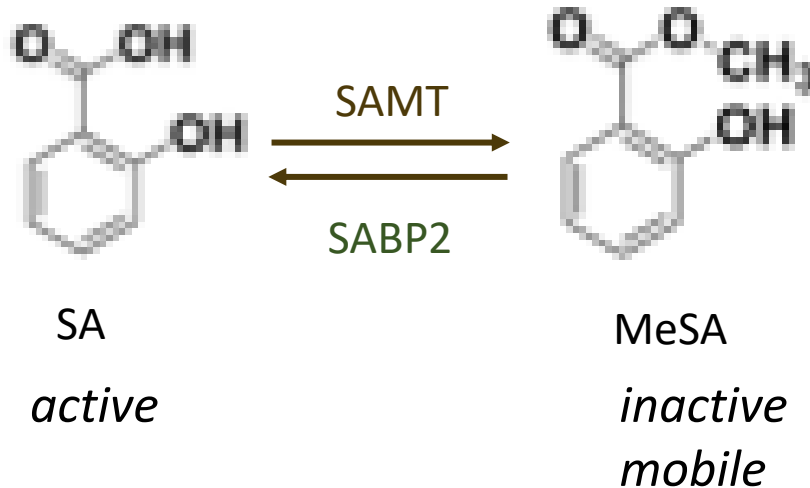
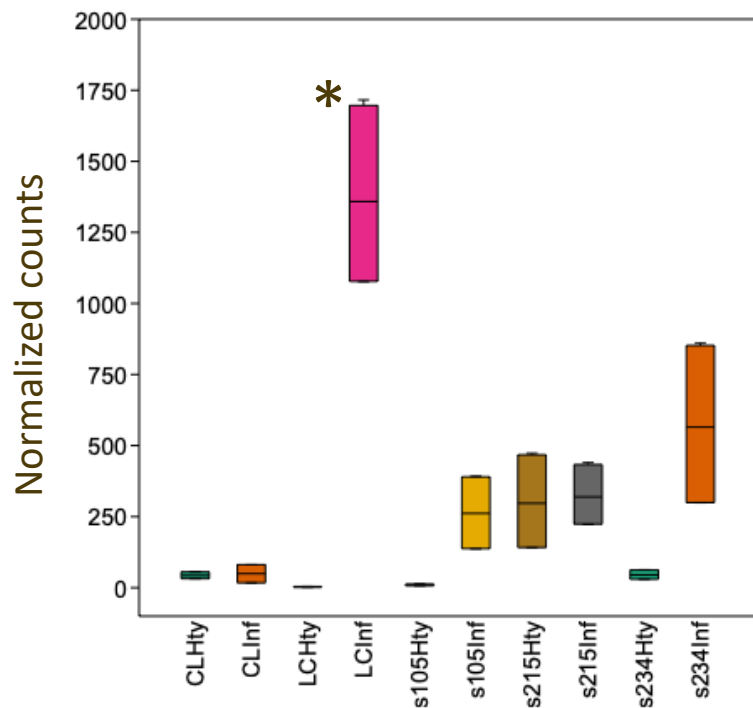
Leccino	5.3
Cellina	2.9



WHAT (likely) MAKES LECCINO RESISTANT?

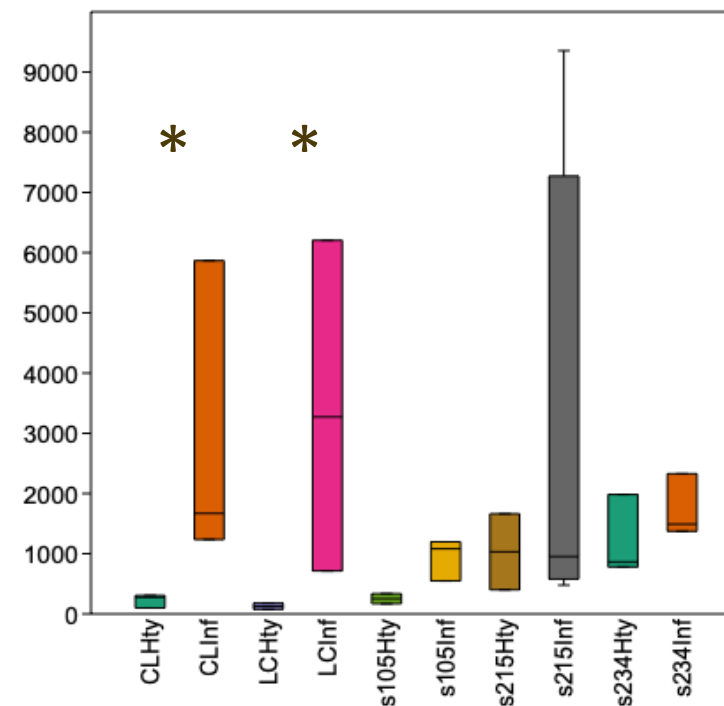
salicylate carboxymethyltransferase-like
OE9A062360, OE9A112830

SAMT



salicylic acid-binding 2,
OE9A038228

SABP2



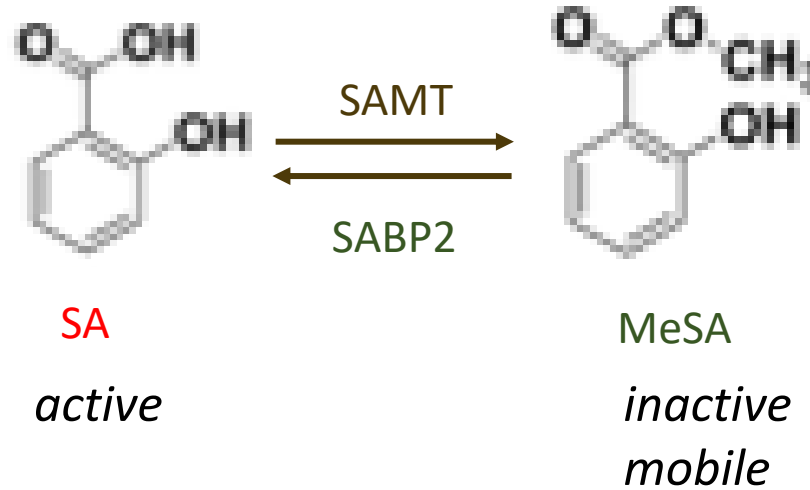
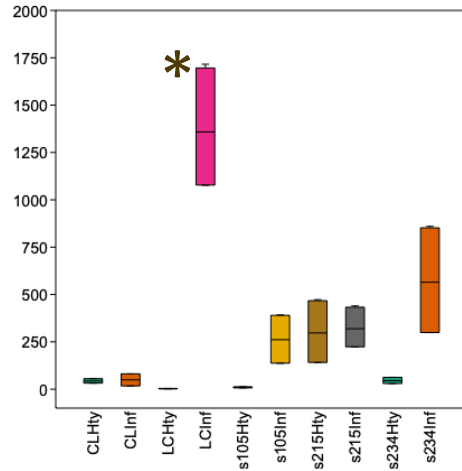
Systemic Acquired Resistance (SAR)

WHAT (likely) MAKES LECCINO RESISTANT?

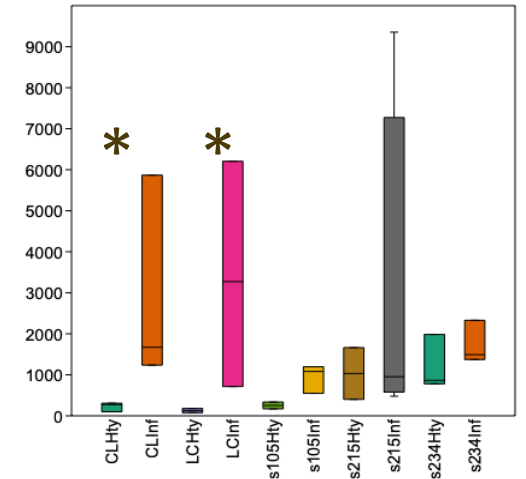
salicylate carboxymethyltransferase-like

salicylic acid-binding 2,

SAMT



SABP2



- ✓ upon infection Leccino converts SA to MeSA (local inactivation/protection from SA excess, high MeSA production)
- ✓ much more MeSA to diffuse systemically in Leccino
- ✓ both cultivars re-convert MeSA to SA systemically (SABP2)
- ✓ higher ability of Leccino to induce a Systemic Acquired Resistance
- ✓ concurrent (tight) control of SA levels by DMR6 and EG45 in all cultivars

Thank you



***Xylella
fastidiosa***

5th European conference

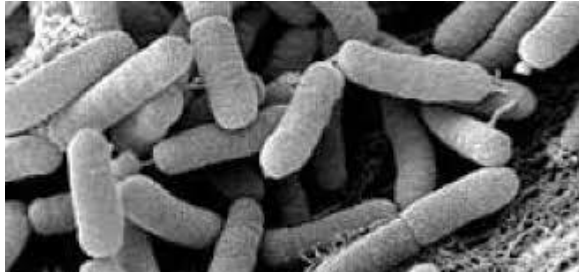
Bari, Italy | 22-26 June 2026

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



REGIONE PUGLIA

Xylella fastidiosa outbreak in Apulia



Xylella fastidiosa outbreak in Apulia

The management of the disease is highly challenging due to several factors, including the rapid transmission of the disease, the broad host range of the pathogen, and the lack of effective treatments.

The most effective control strategy has proven to be the replanting of susceptible olive trees with resistant varieties.

Although significant progress has been made in the comprehension of the mechanism of infection of Xfp in olive and identifying some molecular processes involved in the plant's response to the infection, the specific genes responsible for the resistance to the pathogen have not been identified yet.





Aims of the work

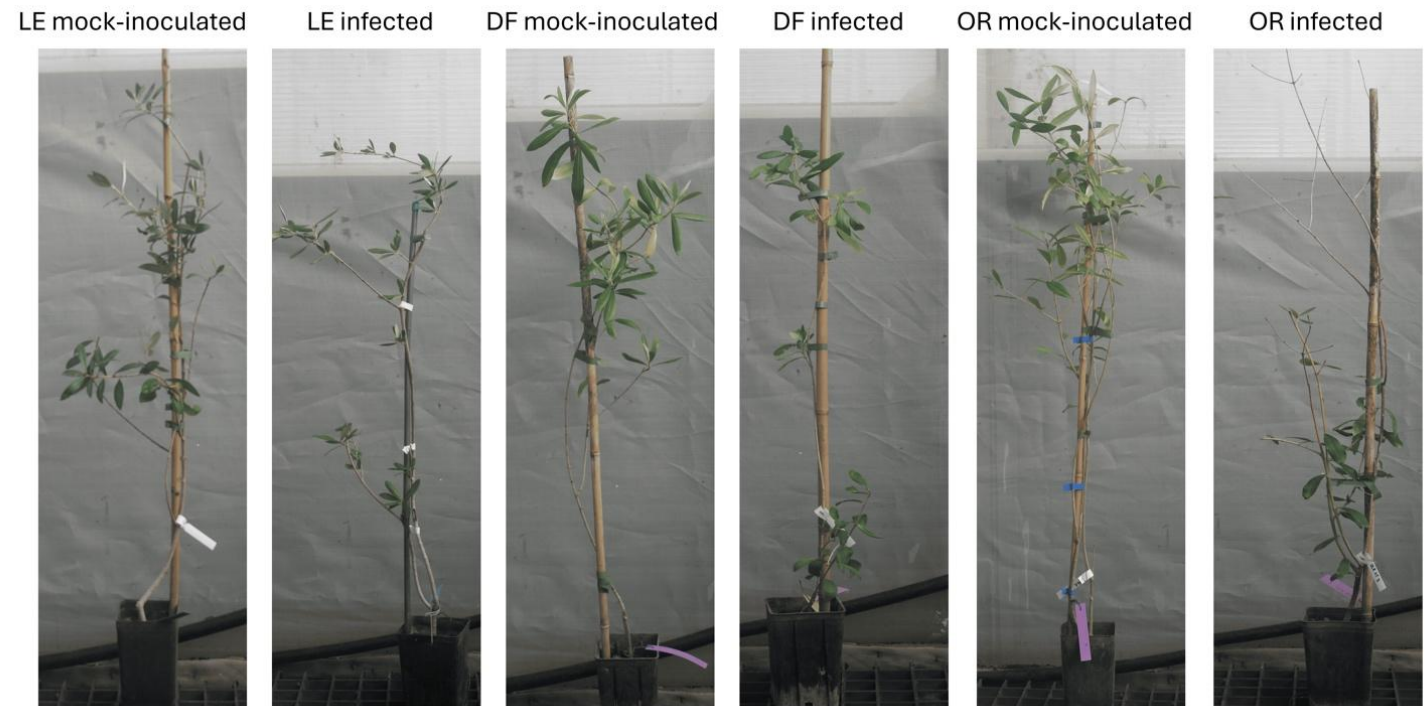
- Comparison of the gene expression across three olive cultivars with distinct responses to *Xylella fastidiosa*: the resistant cultivar LECCINO, the susceptible cultivar OLIVA ROSSA, and moderately susceptible cultivar DONNA FRANCESCA
 - Exploration of the molecular mechanisms underlying host responses by investigating the differentially expressed genes
 - Identification of a list of candidate genes putatively involved in the plant response to the pathogen
- 

Plant material and artificial infection

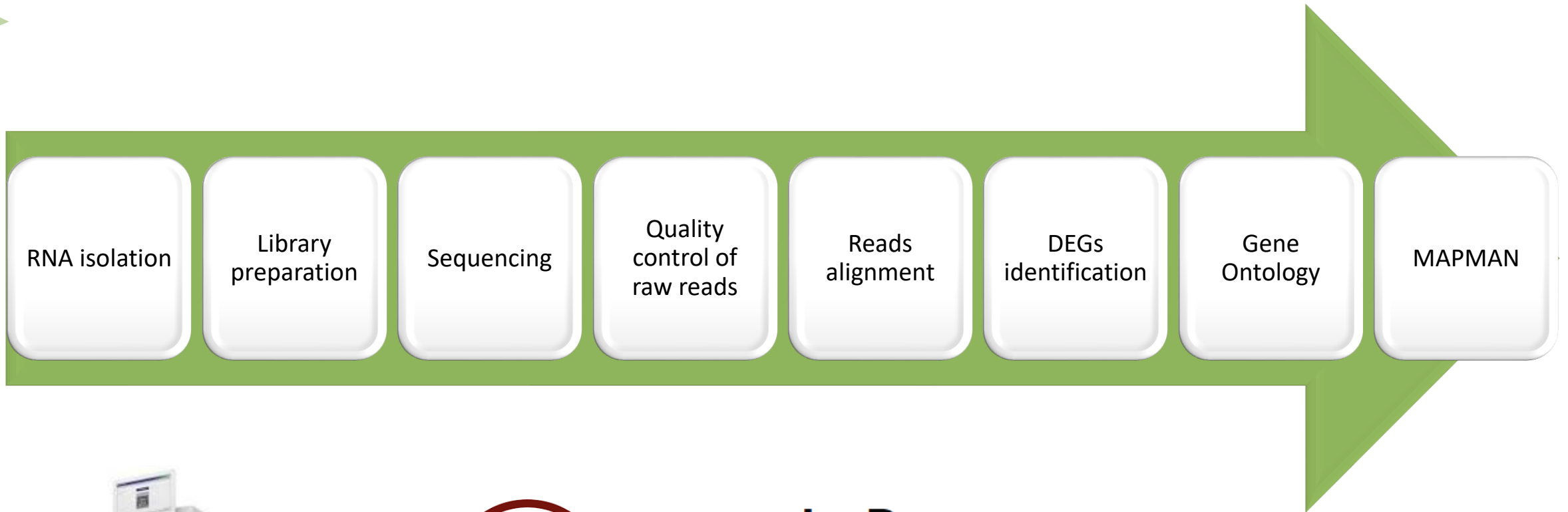
Three plants of each cultivar were inoculated with *Xylella fastidiosa* bacterial suspension and compared with mock-inoculated plants.

Population size of the bacteria was estimated in artificially infected olive plants and the symptom severity was visually assessed.

Leaf and petiole tissues were sampled for RNA isolation.



Sequencing and data analysis



edgeR

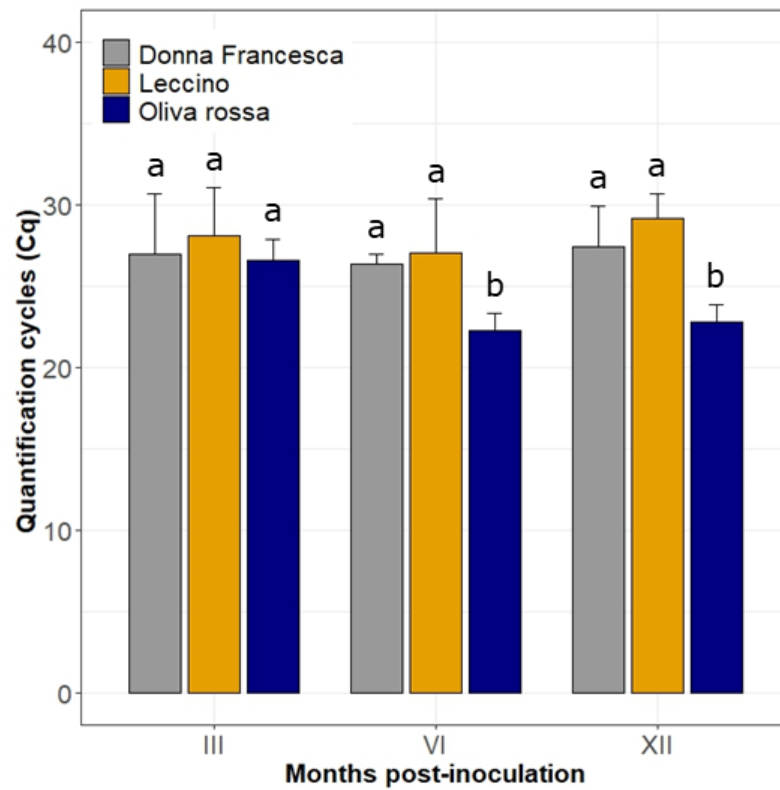
DESeq2

BiNGO

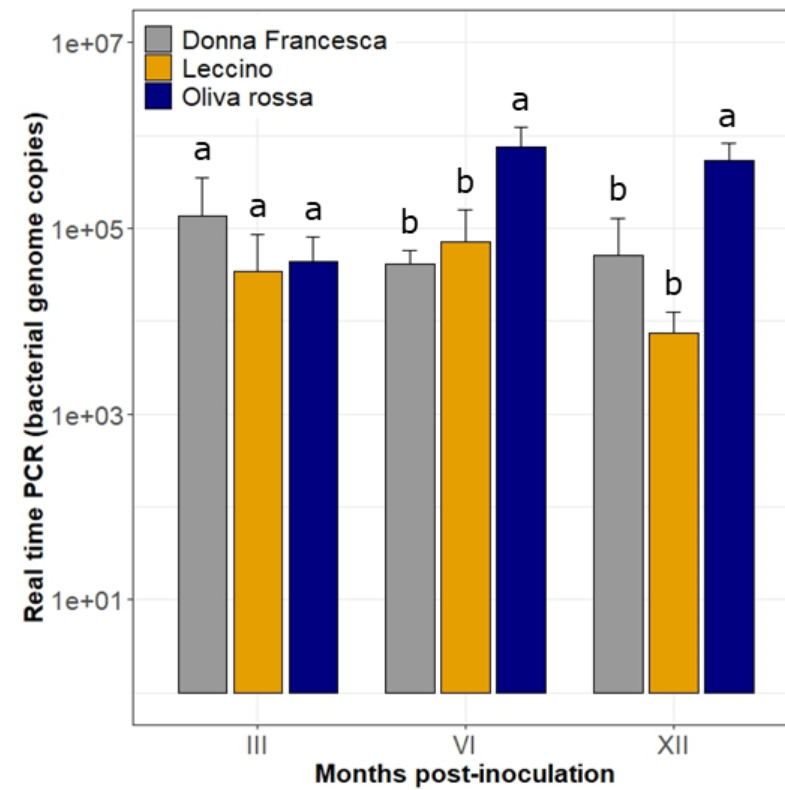


Xylella fastidiosa infection

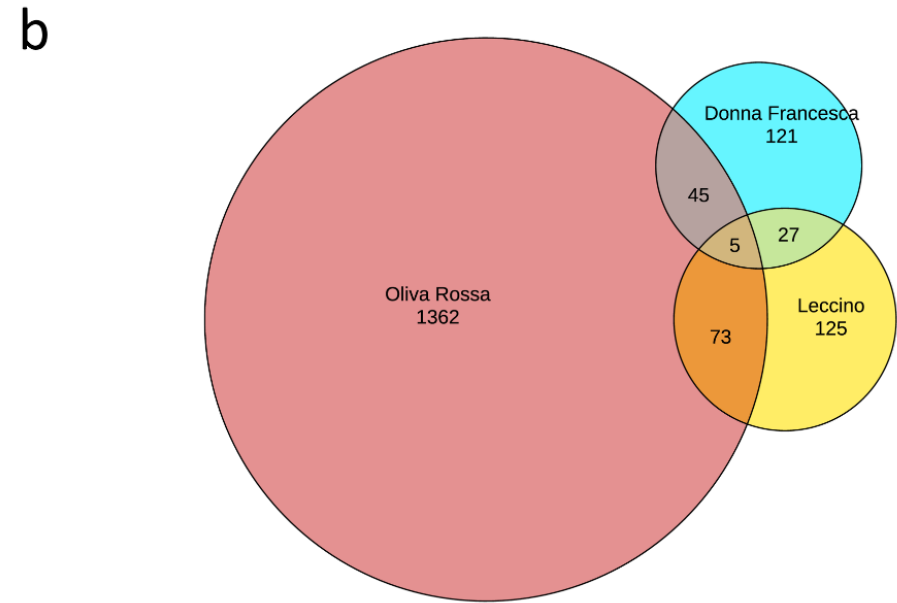
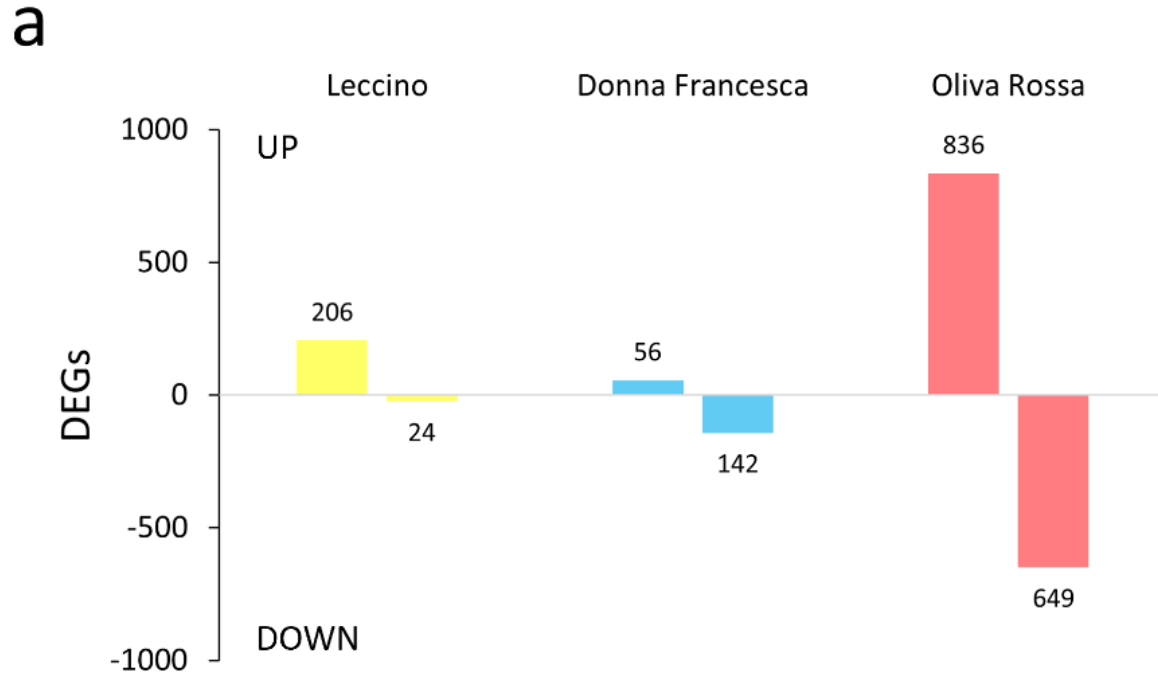
a



b



Gene expression changes triggered by the infection

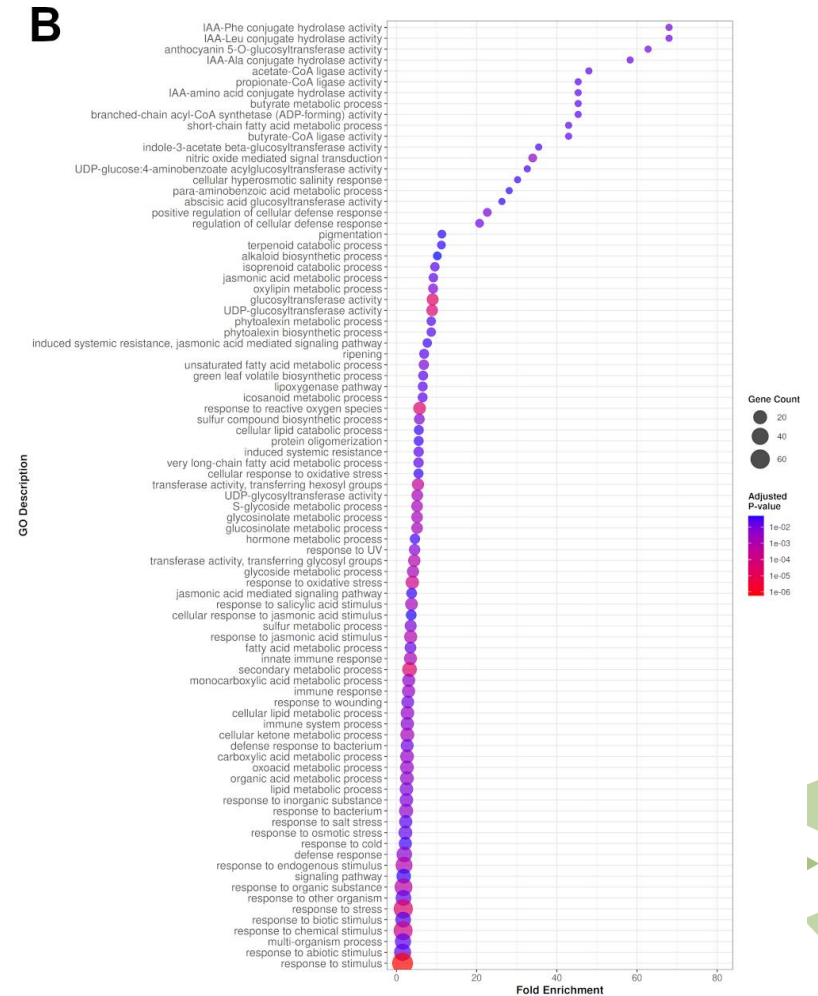
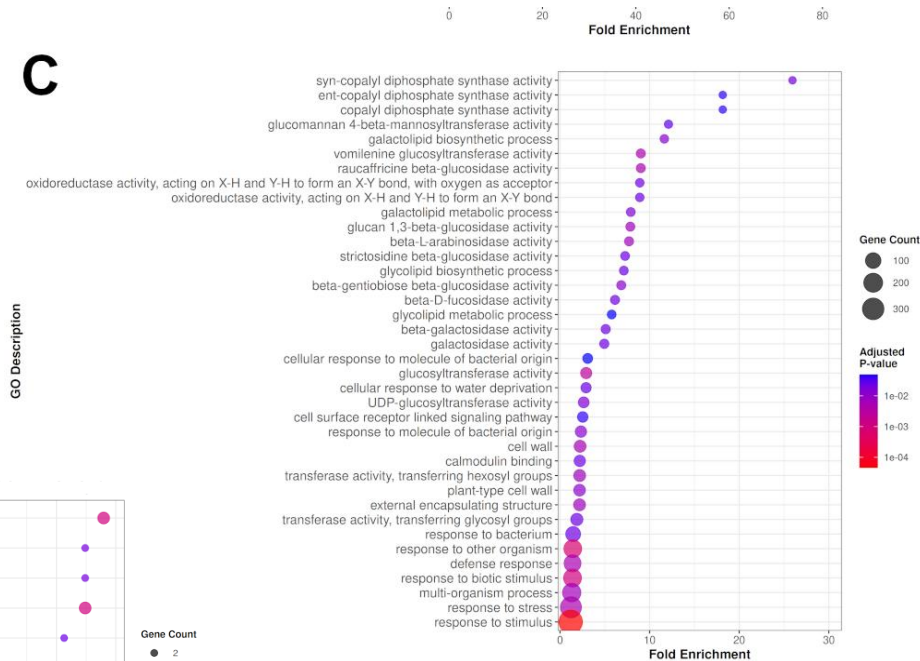
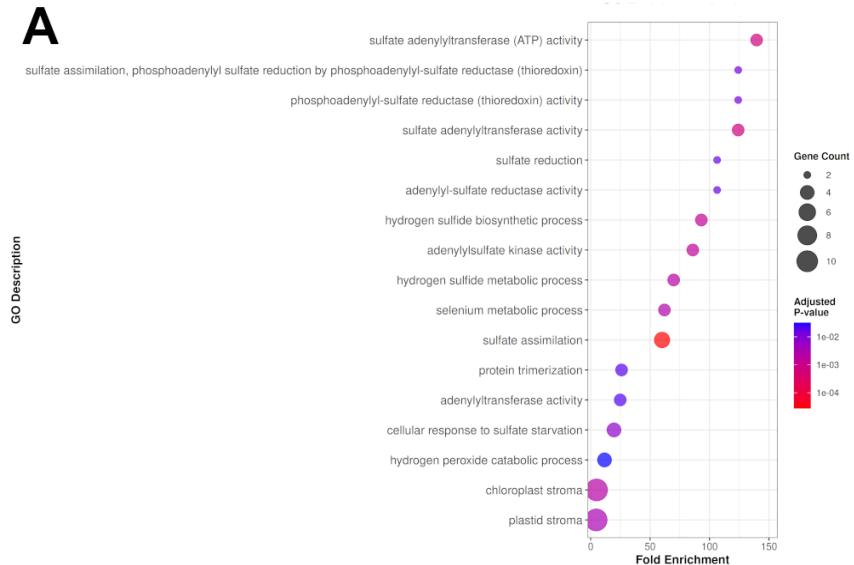


Functional annotation and pathway enrichment analysis of DEGs

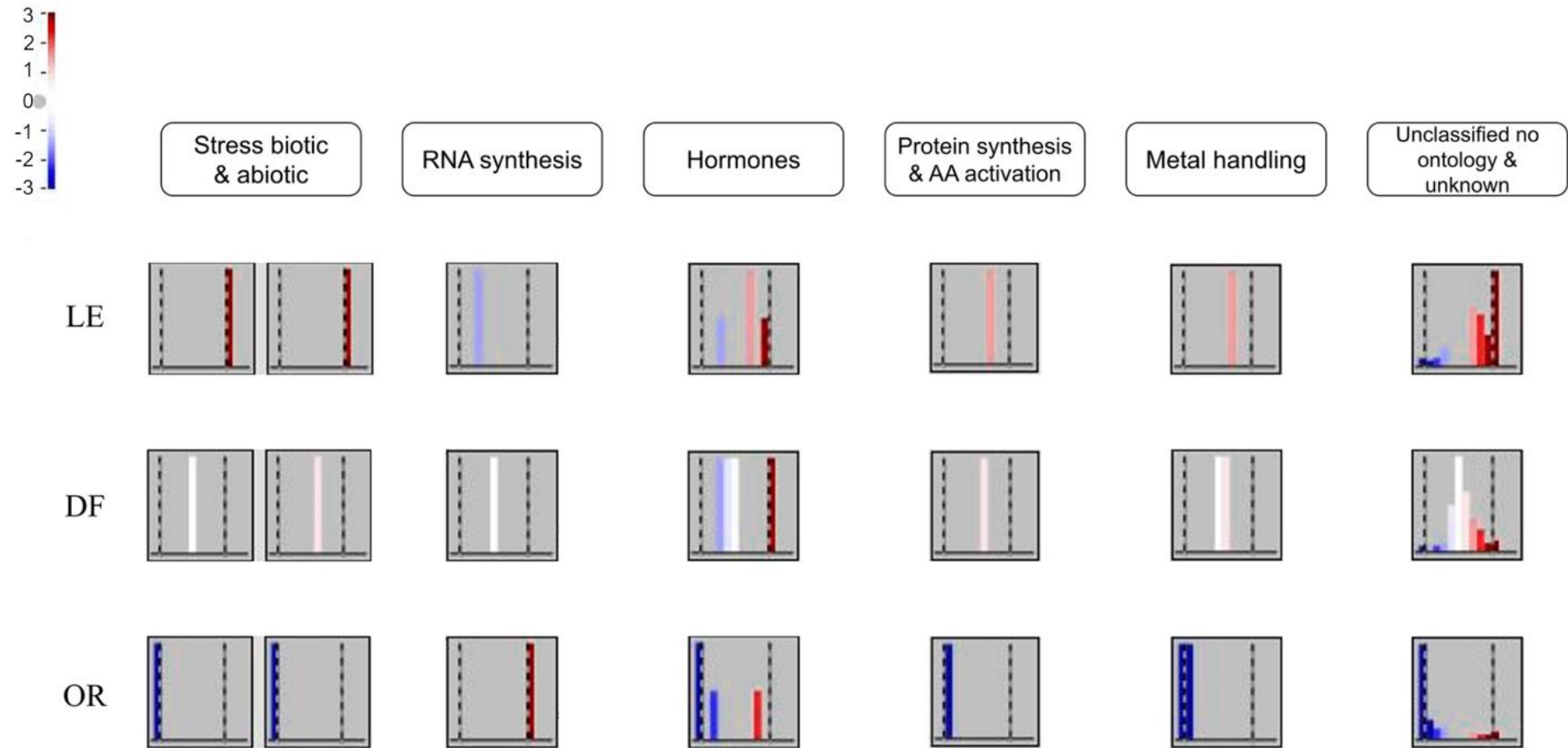
OLIVA ROSSA (upregulated gene set)

DONNA FRANCESCA
(downregulated gene set)

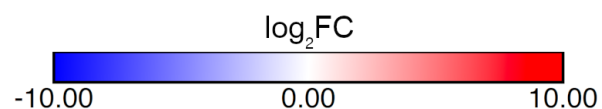
LECCINO
(upregulated gene set)



Functional annotation and pathway enrichment analysis of DEGs



Validation of RNAseq data and identification of candidate genes

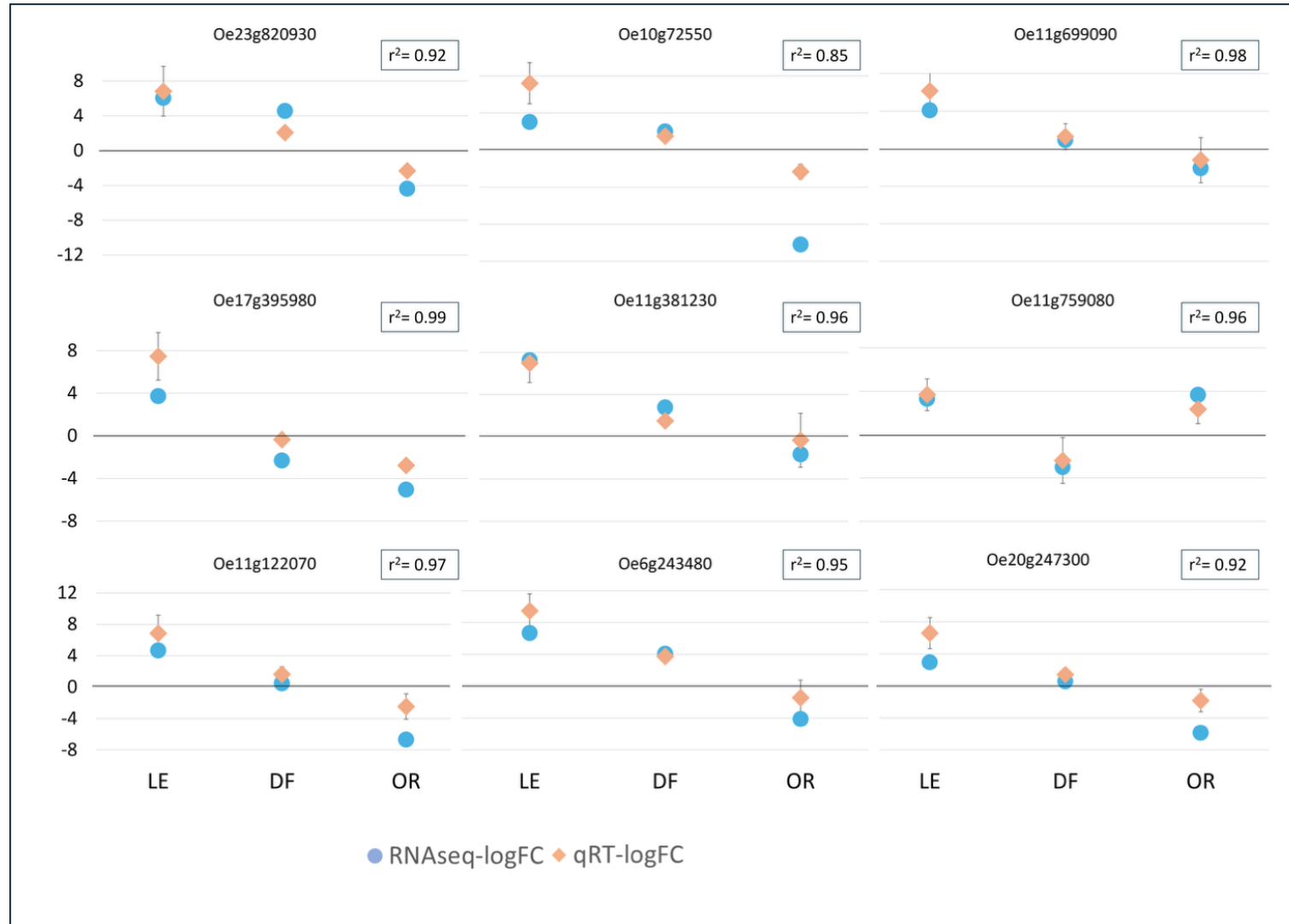


LE DF OR GENE ID

7.25	2.80	-1.65	Oe11g381230	Gene of unknown function
6.04	4.56	-4.37	Oe23g820930	Heavy metal-associated isoprenylated plant protein 7
4.69	2.07	-6.11	Oe6g243480	Retrovirus-related Pol polyprotein, transposon RE1
4.51	-0.01	-7.44	Oe11g122070	Putative disease resistance protein RGA3
4.07	0.91	-2.09	Oe11g699090	Heavy metal-associated isoprenylated plant protein 6
3.73	-2.31	-5.05	Oe17g395980	WAT1-related protein
3.30	-3.01	3.67	Oe11g759080	EG45-like domain containing protein
3.04	2.00	-10.20	Oe10g72550	Gene of unknown function
2.94	0.54	-5.87	Oe20g247300	Noroxomaritidine synthase 2

Gene ID	Chromosome location	Functional annotation
tig02155495g820930	23	Heavy metal-associated isoprenylated plant protein 7
tig00002934g243480	6	Retrovirus-related Pol polyprotein from transposon RE1
tig02154341g759080	11	EG45-like domain containing protein
tig00005818g395980	17	WAT1-related protein
tig00000591g72550	10	na
tig000055272g381230	11	na
tig000011321g122070	11	Putative disease resistance protein RGA3
tig021540532g699090	11	Heavy metal-associated isoprenylated plant protein 6
tig000030141g247300	20	Noroxomaritidine synthase 2

Validation of RNAseq data and identification of candidate genes





Conclusions

- The comparative transcriptomic analysis of olive cultivars with differing susceptibilities to pathogen infection revealed distinct patterns of gene regulation and defence strategies
 - The susceptible OLIVA ROSSA exhibited extensive transcriptional reprogramming, however, these responses were largely ineffective.
 - The moderately susceptible DONNA FRANCESCA showed a restrained transcriptomic response, with weak activation of classical defence pathways indicating that the intermediate level of susceptibility observed is likely mediated by constitutive defence traits.
- 



Conclusions

- The resistant LECCINO demonstrated controlled and highly targeted transcriptional reprogramming, achieving an effective and precise defence response while minimizing collateral disruption to cellular processes.
 - A subset of genes with a diverse response in the three cultivars was identified and they are now being functional validated in a model species.
 - Each variety appears to activate and suppress distinct gene sets upon infection, indicating that resistance and susceptibility in olive arise from fundamentally different molecular strategies. Expanding the analysis to additional cultivars could further clarify the mechanisms underlying these diverse responses.
- 

Conclusions



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Differential transcriptomic landscapes of olive cultivars facing *Xylella fastidiosa* infection

Research | [Open access](#) | Published: 28 May 2026

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Sections

[Abstract](#)

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Approcci integrati per il miglioramento genetico, la
selezione e l'ottenimento di materiali vegetali resistenti a
Xylella fastidiosa



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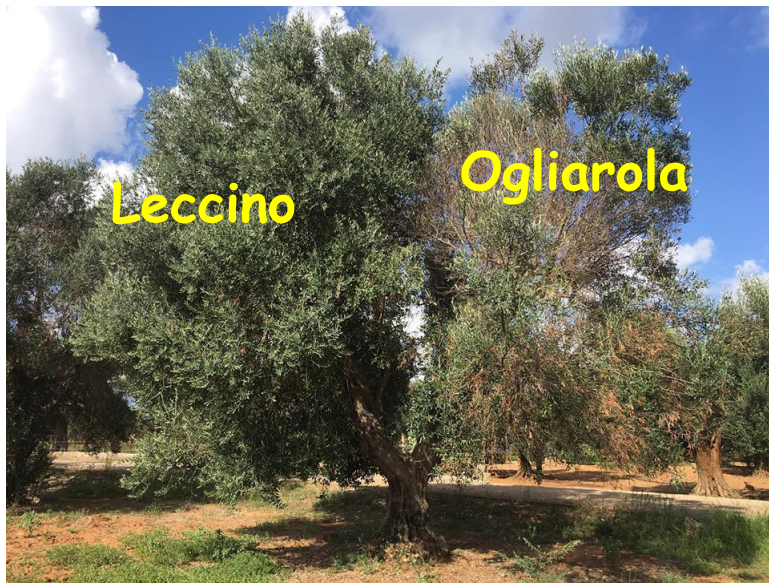


TOP-GRAFTING ANCIENT OLIVE TREES AS PREVENTIVE MEASURE AGAINST OLIVE QUICK DECLINE SYNDROME: PRELIMINARY FIELD EVIDENCE

*Saponari M., La Notte P., Melcarne G., Pugliese G., Zigrino M., Ligorio A.M., Leone
C., Zaccaria L., Specchia F., Boscia D.*

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To preserve ancient olive trees from the severe impact of *Xylella fastidiosa* subsp. *pauca* ST53, attempts are made to **substitute the canopy of susceptible trees by top-grafting scions of the resistant cultivars**

Top grafts carried out on already sick trees in the first years of the epidemic had a high percentage of failures, however observations of some grafts of Leccino carried out casually before the epidemic and in good vegetative state have led to the hypothesis that **grafting as a preventive action could help to safeguard valuable monumental olive trees**, this is why **the Puglia Region funds grafting** interventions with scions of olive resistant

A survey was conducted between late 2025 and early 2026 in four sites in the province of Brindisi (Apulia, Italy), currently included in the demarcated infected but on the forefront of the epidemics and under high pressure of inoculum

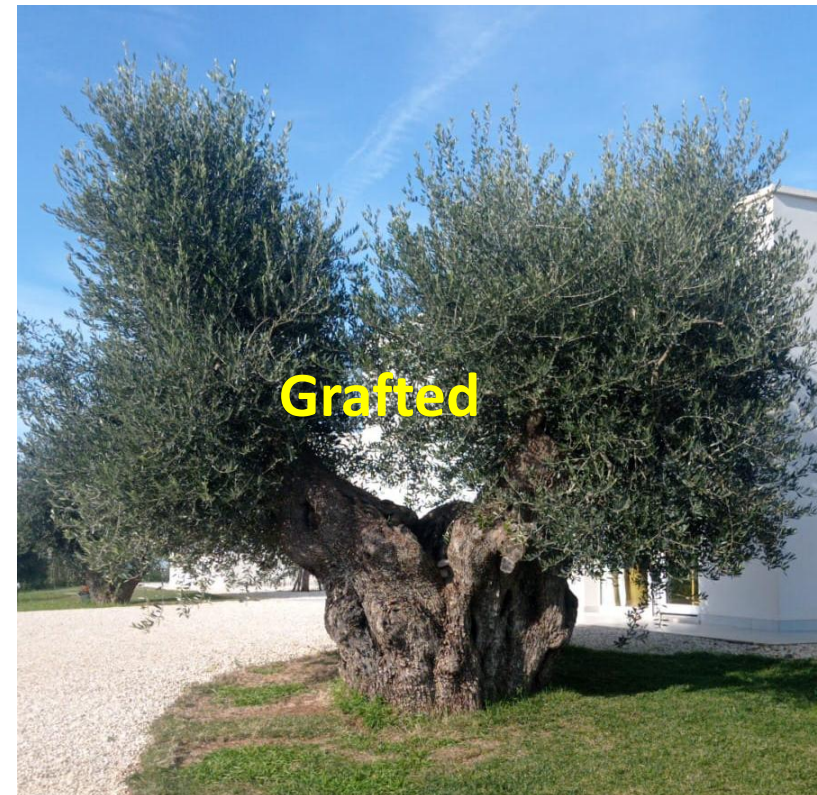


Grafts were performed between 2021 and 2023, when most of the trees were still asymptomatic, although some may have already been infected

- 
- The majority of the trees top-grafted showed vigorous canopies and no sign of bacterial infections, whereas trees in neighboring groves exhibited severely decimated canopies affecting 100% of the trees.
 - Laboratory analysis revealed a percentage of grafted trees positive to Xylella ranging from 0 to 50%, while all the surrounding non-grafted trees were infected (100%)
 - occasionally some trees (approx. 13%) developed symptoms, always correlated to the occurrence of infections in the cultivar OG, thus suggesting infections were already established before grafting. Nevertheless, the percentage of **symptomatic** trees in the **top-grafted** groves was significantly **lower** than in the neighboring groves (100%), with **symptoms being markedly less severe**

Taken together, these preliminary observations support the assumption that **grafting with resistant cultivars performed before infections can help to preserve and protect ancient, monumental olive trees**





A photograph of an olive grove with several large, mature olive trees. The trees are situated in a field of tall, green grass. The sky is blue with some white clouds. The trees are labeled 'Grafted' and 'Non grafted' in yellow text. The grafted trees are on the left, and the non-grafted trees are on the right. The grafted trees have a more compact, rounded canopy, while the non-grafted trees have a more spread-out, irregular canopy. The trunks of the trees are thick and gnarled, indicating their age. The overall scene is peaceful and rural.

Grafted

Non grafted



Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026



REGIONE PUGLIA

THE WORLD OLIVE GERmplasm BANK OF CORDOBA: A SOURCE FOR RESEARCH ON XYLELLA CONTROL

- ✓ Maintained at IFAPA (Córdoba): **55** years
- ✓ National and international referent collection
- ✓ ≈ **1300** accesions : **29** countries (**15** ha)
- ✓ Accurately identified: **775** cultivars
- ✓ Backup collection IFAPA (Jaén)

Diversity-Based Cultivar Selection

≈ **80** cultivars: **20** countries

Xf Controlled and field screening trials

Ongoing evaluation: Italy and Mallorca

Identify tolerant/resistant cvs to Xf



A. Belaj, L. León, R. de la Rosa

IFAPA

Instituto de
Investigación
Formación Ag
y Pesquera

IAS  **CSIC**

THE WORLD OLIVE GERmplasm BANK OF CORDOBA: A SOURCE FOR RESEARCH ON XYLELLA CONTROL

Breeding for Xylella Resistance Using WOGBC Diversity

Progenies of Xf tolerant cultivars

195 seedlings

'Leccino' x 'Favolosa'

'Leccino' o.p.

'Favolosa' o.p

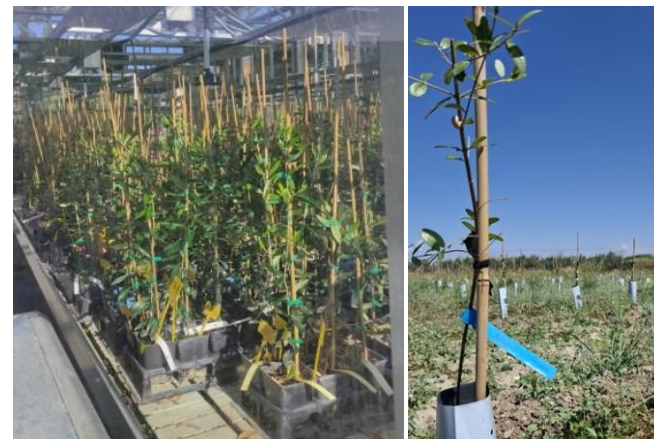


- ❖ **Long juvenile period:** only 28% < 5 years
- ❖ **Only 9 genotypes** (4.6%) fulfilled both criterion of low juvenility & oil content (≥ 40 % in dry matter)
- ❖ **Testing of this set of genotypes** under both controlled and field conditions **for Xf resistance in Italy and Mallorca.**

Progeny: Xf resistance & agronomic traits

280 seedlings

'Arbosana' x 'Leccino'



- ❖ **Ongoing field evaluation of the progeny**

THE WORLD OLIVE GERmplasm BANK OF CORDOBA: A SOURCE FOR RESEARCH ON XYLELLA CONTROL

Thermotherapy Evaluation in WOGBC Cultivars

Plant material

18 cultivars: **13** countries

Treatments

To	Control	
T1	42°C	40 min
T1	42°C	60 min
T2	44°C	40 min
T2	44°C	60 min

Experimental design:

18 cvs x **5** Hwt treatments

4 replicates/each T



- ❖ Most cultivars failed to root under thermotherapy treatment.
- ❖ Few cvs showed very low (6.7-13.3%) rooting rates (mostly at 42°C and 40')

THE WORLD OLIVE GERmplasm BANK OF CORDOBA: A SOURCE FOR RESEARCH ON XYLELLA CONTROL

Cultivar Identification in Xf-Affected Areas

EST-SNP-based identification of olive trees in the Balearic Islands



243 prospected trees

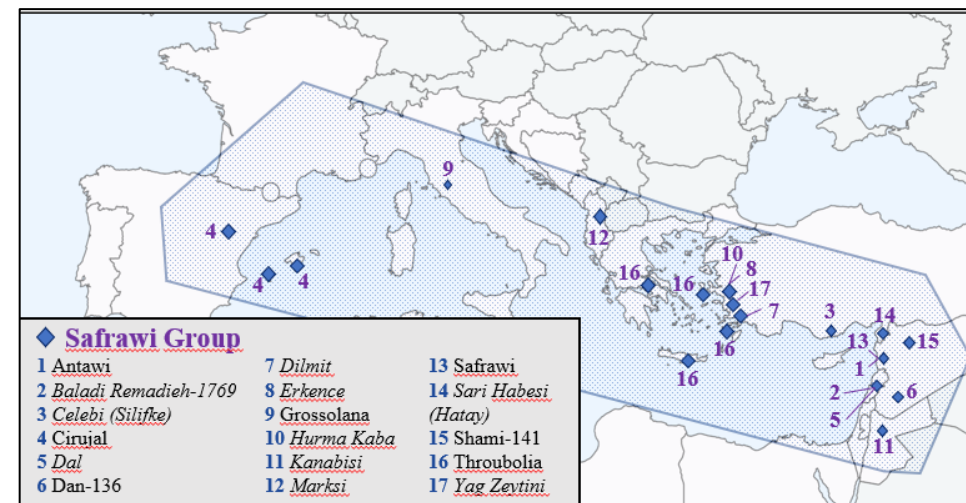
85 different genotypes

54 wild genotypes

31 cultivars



9 unknown local cultivars

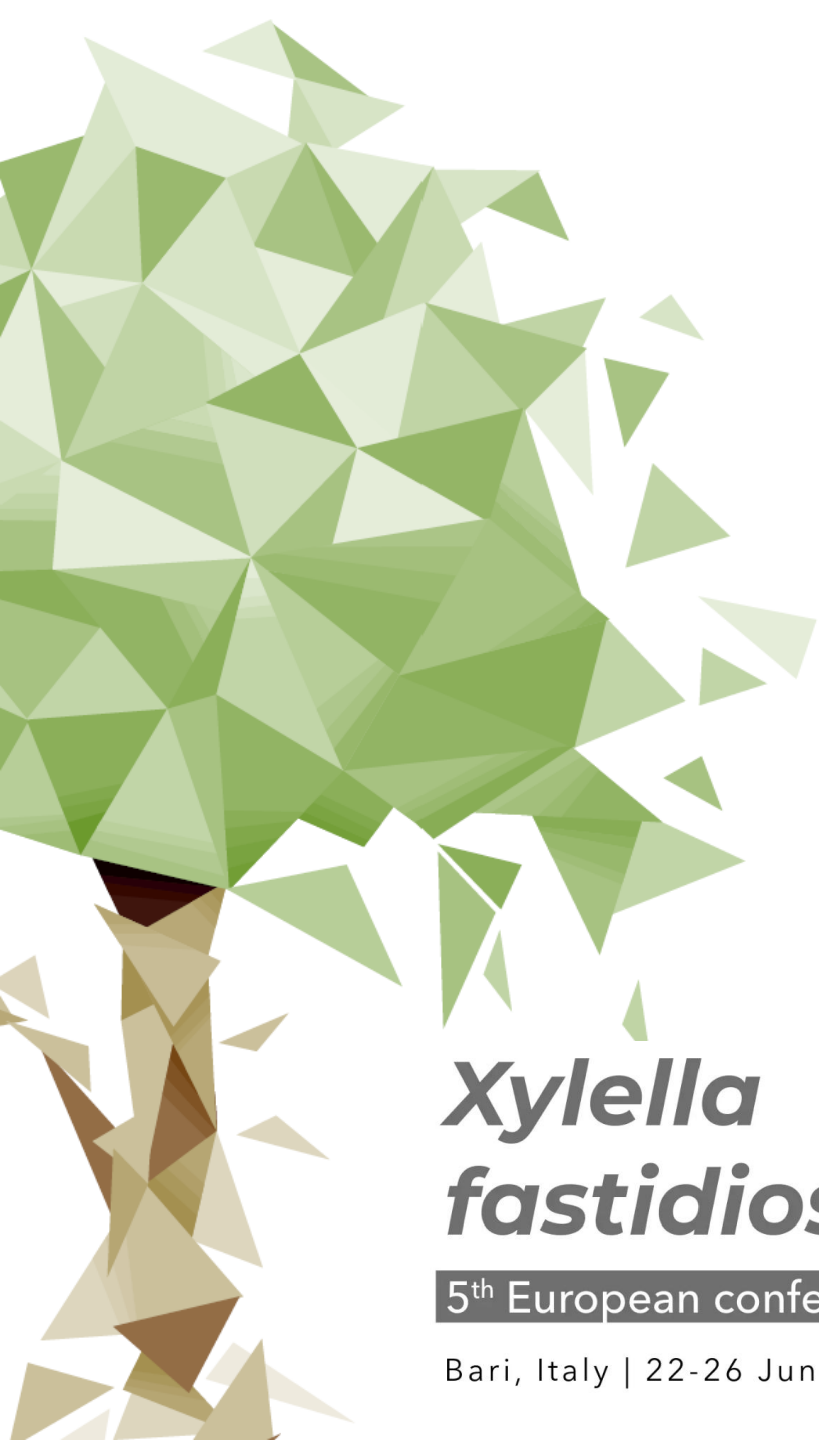


THE WORLD OLIVE GERmplasm BANK OF CORDOBA: **A SOURCE FOR RESEARCH ON XYLELLA CONTROL**

Today's conserved diversity is tomorrow's resilience



Thank you for your attention!



GENOME EDITING IN OLIVE: FROM EMBRYOGENIC CALLUS INDUCTION TO A PROTOPLAST-BASED PLATFORM

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*Xylella
fastidiosa*

5th European conference

Bari, Italy | 22-26 June 2026



REGIONE PUGLIA

URGENT GOALS IN OLIVE RESEARCH

- ADDRESSING THE SPECIES' RECALCITRANCE TO *IN VITRO* AND GENETIC MANIPULATION
- INVESTIGATING REGENERATION TO OVERCOME THIS BOTTLENECK
- CLEAN GENOME EDITING

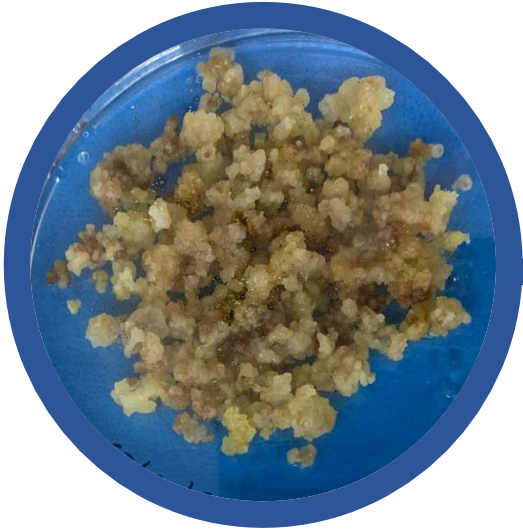


GENOME EDITING

MATERIALS
PROTOPLASTS,
CALLI,
IN VITRO PLANTS.

REGENERATION

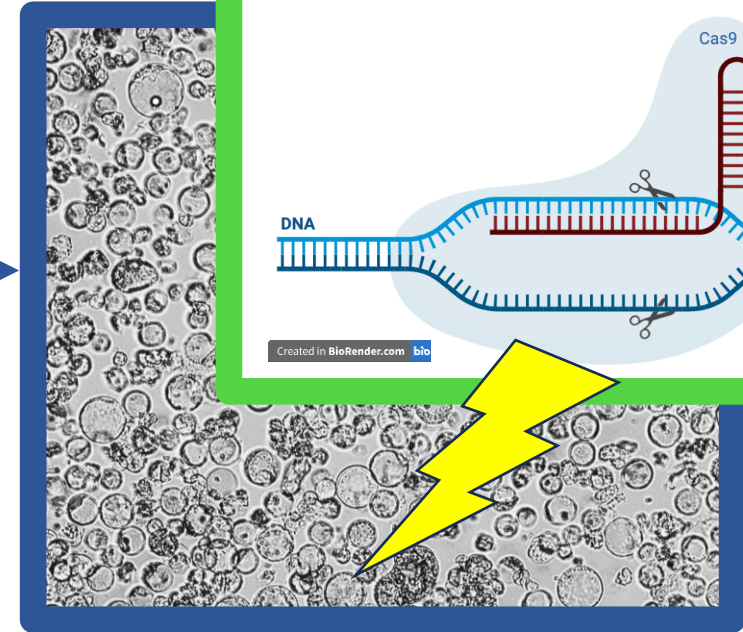
PROTOPLAST-BASED PLATFORM



EMBRYIOGENIC
CALLUS OF
CULTIVAR **PICUAL**

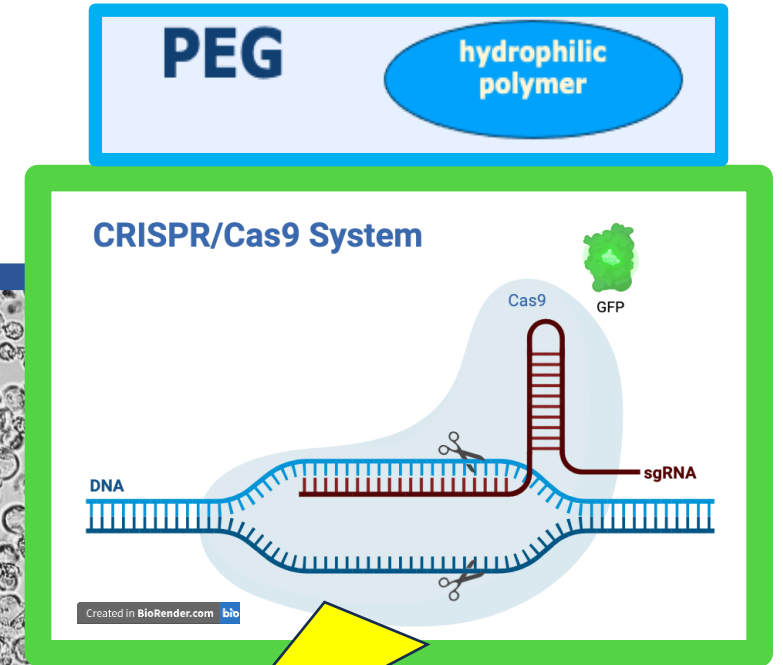


ISOLATION PROTOCOL
(GENOTYPE-DEPENDENT)



PROTOPLASTS

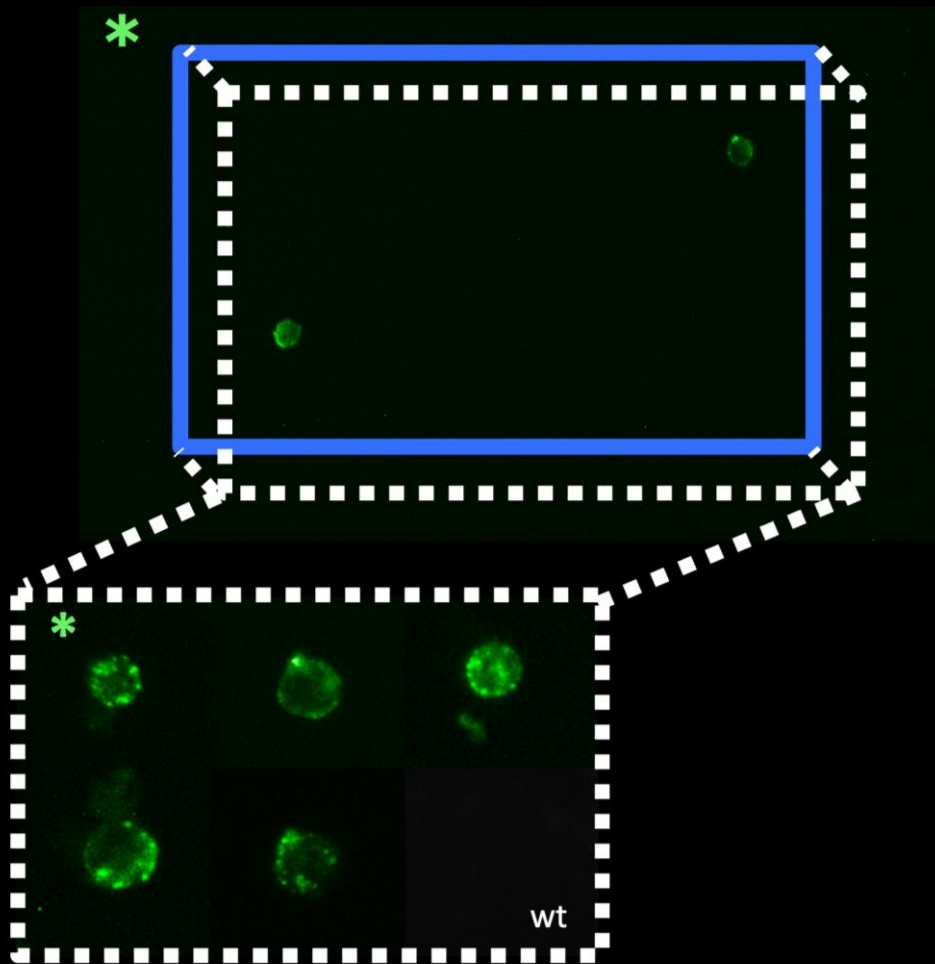
- **NAKED CELLS**
- **EDITING OF SINGLE CELLS**



GENOME EDITING TRIALS

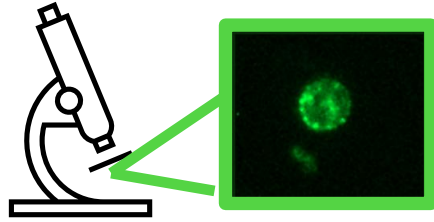
IT'S **FEASIBLE!**

EMBRYOGENIC **C**ALLUS-
DERIVED **P**ROTOPLASTS
(**ECPS**) ARE A PROMISING
PLATFORM FOR
TRANSGENE-FREE GENOME
EDITING IN **OLIVE**.



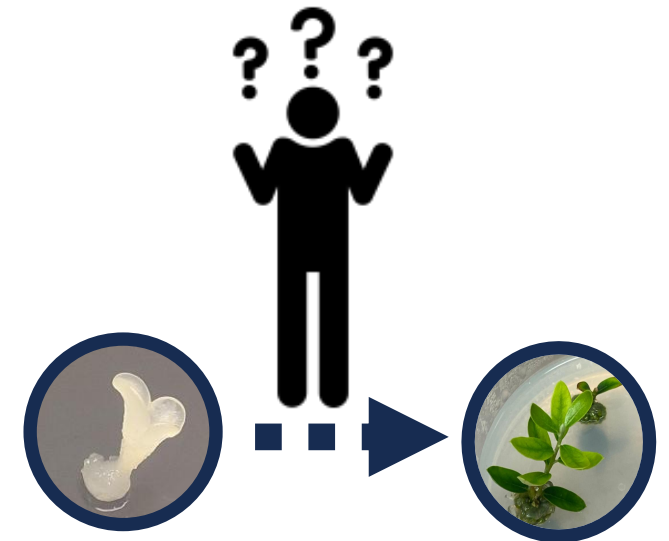
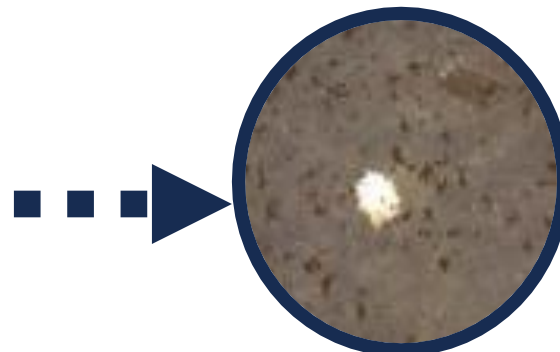
* REPRESENTATIVE PHOTOS OF FLUORESCENT ECPS FROM PICUAL'S **CALLUS** IN COMPARISON TO WILD-TYPE (WT) AT THE FLUORESCENCE MICROSCOPE.

REGENERATION TRIALS



THE ENTRANCE OF THE EDITING SYSTEM IS A VALUABLE PROOF OF CONCEPT.

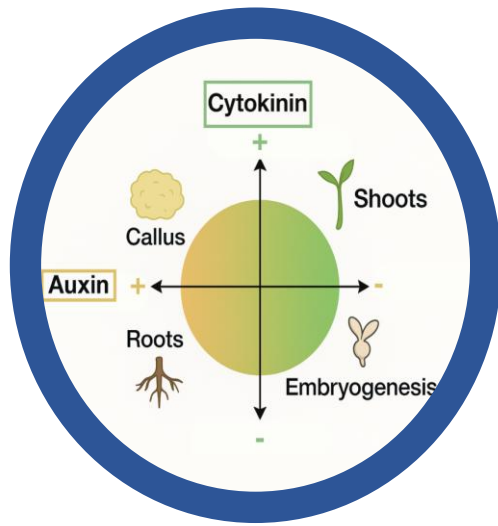
ALTHOUGH, CULTURING PROTOPLASTS IS NECESSARY TO ASSESS THEIR ABILITY TO DEVELOP.



**PROGRESS TOWARDS
WHOLE-PLANT
REGENERATION ?**



EMBRYOGENIC CALLUS INDUCTION IN OTHER OLIVE CULTIVARS (CV.)



PICUAL



SEED-DERIVED

FAVOLOSA



KALAMATA



T

S



ADULT EXPLANTS

SEED-DERIVED EMBRYOGENIC CALLUS OF CV. PICUAL USED AS A REFERENCE MODEL FOR THE EVALUATION OF MORPHOLOGICAL FEATURES SHOWED BY CALLI INDUCED FROM ADULT EXPLANTS OF CV. FAVOLOSA AND KALAMATA, RESPECTIVELY TOLERANT (T) AND SUSCEPTIBLE (S) TO *XYLELLA FASTIDIOSA*.

FUTURE PURPOSES :

- ENRICHING THE PROTOPLAST-BASED PLATFORM WITH THESE NEW CV.
- EDIT GENES CONTRIBUTING TO THE DEFENSE RESPONSE AGAINST PATHOGENS.

DEVELOPMENT OF EFFECTIVE EMBRYOGENIC CALLUS INDUCTION AND REGENERATION PROTOCOLS ADDRESSED TO SPECIFIC OLIVE CULTIVARS

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INTRODUCTION

Embryogenic callus represents a highly valuable starting material for a wide range of biotechnological applications; however, its induction in olive (*Olea europaea* L.) remains challenging. Not all tissues can form it, and regeneration protocols are largely genotype-dependent. As a result, different cultivars often exhibit highly variable responses to the same culture media and conditions. Therefore, precise control of timing and culture conditions is essential throughout the process, particularly about the correct application and adjustment of plant growth regulators (PGRs).

To date, only few protocols are available, and fewer starting from mature explants.

Efficient induction of embryogenic calli from adult explants remains a key bottleneck for the application of the New Genomic Techniques (NGTs) applied in this species, yet it's essential as a starting material for generating more tolerant and/or resistant varieties to pathogens affecting the species, including *Xylella fastidiosa*.



METHODOLOGY

The induction and maintenance of embryogenic calli is being evaluated in cultivars (cv.) with different responses to *Xylella fastidiosa*: the tolerant cv. **Favolosa** and the susceptible cv. **Kalamata**. Leaves, shoot apices and petioles from young *in vitro*-derived sterile shoots of the cultivars were cultured onto solid or liquid media containing different PGRs combinations.

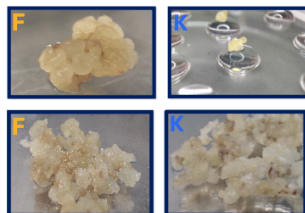
The obtained calli are being cultured on **ECO Medium** (Pérez-Barranco et al., 2009 - Plant Cell Tiss Organ Cult 97: 243-251) and newly developed culture media, showing improved growth and survival rates. Testing compounds such as silver nitrate (AgNO₃), has shown to alter the morphology of the callus tissue, promoting in some cases, embryogenic-like growths.

RESULTS

Various protocols have led the formation of **friable calli**, shown in the figure.

Both cultivars' calli induced from adult tissues show pre-embryogenic features. These observation are encouraging for future improvement.

At this stage, generation of **somatic embryos** is still missing, although regeneration trials are still ongoing.



Friable calli of Favolosa (F) and Kalamata (K) at different stages.

CONCLUSIONS

The **next step** will be the regeneration of somatic embryos in the coming months.

Achieving this milestone will enable the effective use of calli derived from the cultivars described above as a platform for subsequent applications, including transgene-free genome editing (GE). In the long term, this approach is expected to lead to the generation of edited olive plants carrying desirable agronomic features.

Ultimately, the development of a robust regeneration and transformation framework will contribute to overcoming one of the major bottlenecks in olive biotechnology, and accelerating the improvement of traits relevant to productivity, resilience, and sustainability in this species.



GENOME EDITING IN OLIVE: FROM EMBRYOGENIC CALLUS INDUCTION TO A PROTOPLAST-BASED PLATFORM

Giorgia Sportelli^{1*}, Mario Nicotera¹, Laia Ribalata Campos², Roberta Spanò¹, Annalisa Giampetruzzi¹, Pasquale Saldarelli¹, Fernando Pliego-Alfaro², José A. Mercado², Maria Saponari¹, Giorgio Gambino¹, Irene Perrone¹
¹Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche (CNR-IPSP) Torino and Bari, Italy;
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INTRODUCTION

Embryogenic callus-derived protoplasts (ECPs) represent a promising platform for the development of **transgene-free genome editing (GE) in olive** (*Olea europaea* L.). This approach is particularly valuable in woody crops where long juvenile phases, extended generation times, and complex genetic backgrounds are critical points. Consequently, GE offers a precise, fast, transient, versatile, multiplex and relatively inexpensive, strategy to the genetic improvement in this species.

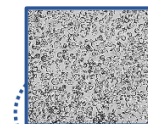
METHODOLOGY

Embryogenic callus from cultivar (cv.) **Picual** is mechanically fragmented and subjected to enzymatic digestion to release protoplasts which must be purified, stabilized, washed, and counted. After the evaluation of several transfection buffers, **MMG solution** has been selected as the primary transfection medium in combination with polyethylene glycol (PEG), a hydrophilic polymer that transiently increases membrane permeability and facilitates the uptake of exogenous molecules into cells.

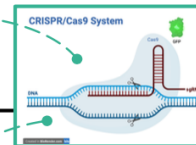
Using a PEG-mediated delivery approach, **CRISPR/Cas9** editing system, consisting of Cas9 protein and guide RNA, is introduced as ribonucleoproteins (RNPs) complexes. These RNPs knock-out selected genes and are naturally degraded by the cell after performing their function.



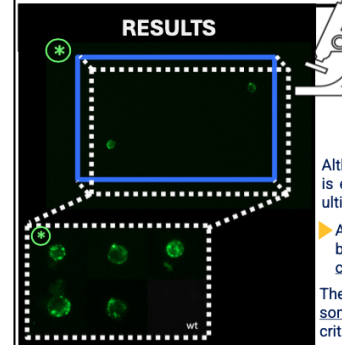
From embryogenic callus of cv. Picual...



...to protoplasts.



RESULTS



The fluorescence of GFP conjugated with Cas9 confirms the entrance of the editing system, as shown in the representative photos of **fluorescent ECPs from cv. Picual** in comparison to wild-type (wt) at the fluorescence microscope, demonstrating the **feasibility of GE** through the delivery of RNPs directly into cells.

Although this valuable proof of concept for GE in olive, **protoplast culture** is essential to assess cells' competence to go towards regeneration and ultimately generate whole edited plants.

At present, somatic embryos development has not yet been achieved, although regeneration experiments are currently in progress.



The development of efficient regeneration systems supporting cell division, **somatic embryogenesis**, and subsequent **plant recovery** is therefore a critical prerequisite for the large-scale application of GE in this crop.



In parallel, to **enrich the ECPs platform** with new starting material, the induction and maintenance of new embryogenic calli is being evaluated in two cultivars with different responses to *Xylella fastidiosa*: the tolerant cv. **Favolosa** and the susceptible cv. **Kalamata**. The **future purpose** is using ECPs from these calli to edit genes contributing to the defense response against *Xylella* and other pathogens, working with **DMR6** (Downy Mildew Resistant 6) gene family and others.

CONCLUSIONS

The olive's **recalcitrance** to *in vitro* regeneration and genetic manipulation is a constraint; however, the full **potential** of this combining technology is not evaluated yet. **Ongoing efforts seek to move beyond these bottlenecks towards more advanced outcomes** which would pave the way for the rapid improvement of olive cultivars while preserving their valuable genetic features.





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Giorgio Gambino



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Mario Nicotera

Roberta Spanò

Annalisa Giampetruzzi

Pasquale Saldarelli

Maria Saponari

Laia Ribalta Campos

Fernando Pliego-Alfaro

Josè A. Mercado



THESE WERE MY FIVE MINUTES.

THANK YOU ALL 😊



Plant Cell Wall Dynamics in Olive Cultivars with Contrasting Responses to *Xylella fastidiosa*

DANIELE COCULO

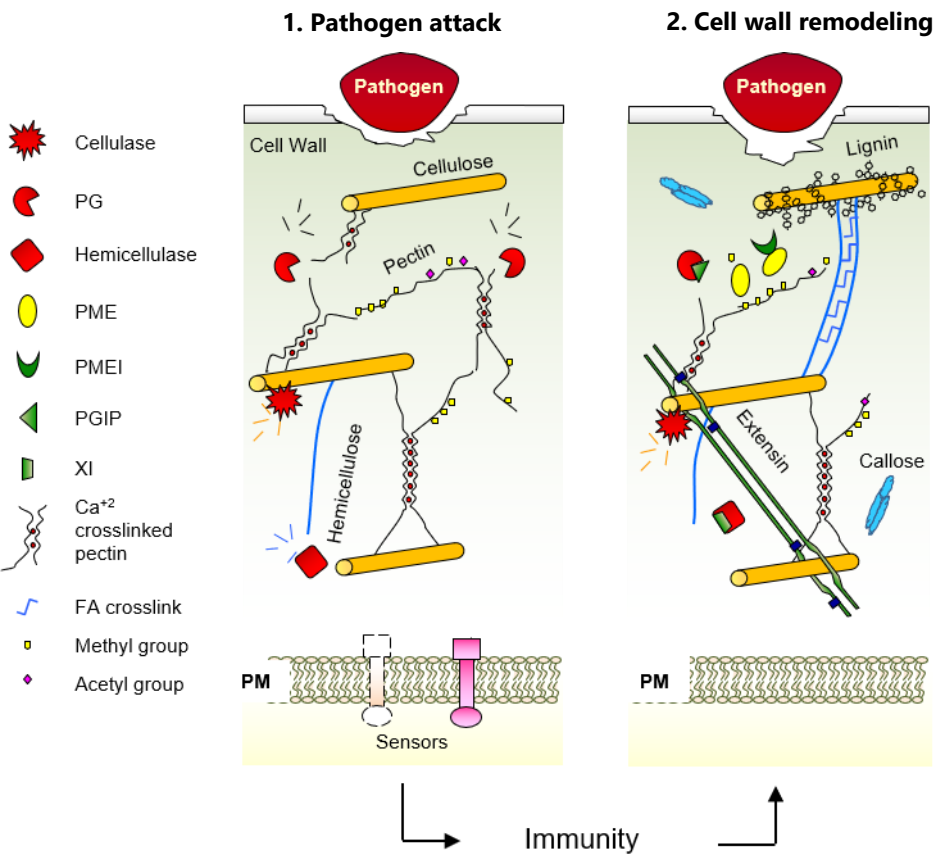


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CAN THE CELL WALL BE A CONTRASTING FACTOR OF *Xylella fastidiosa* COLONIZATION?

Plant cell wall is remodeled during infection



		HG low methylester (LM18)	HG low methylester (LM19)	HG high methylester (LM20)	RGI Backbone (INRA-RU1)	RGI Backbone (INRA-RU2)	RGI Galactan side chains (LM5)	RGI Arabinan side chain (LM6)	(galacto)mannan (BS-400-4)	(galacto)mannan (LM21)	(gluco)mannan (LM22)	Callose (BS-400-2)	Xyloglucan (XXXG - LM15)	Xyloglucan (XLLG - LM24)	Extensin (LM1)	Extensin (JIM11)	Extensin (JIM20)	AGP (JIM13)	AGP, β-linked GlcA (LM2)	AGP (LM14)
Hemicellulose	Mock	26	33	24	24	42	14	23	8	0	0	0	0	0	9	25	34	0	16	31
	Botrytis	10	10	0	17	31	21	15	0	0	0	0	0	0	15	44	52	5	16	31
	Mock	0	8	0	10	10	9	6	49	36	10	10	100	9	8	14	18	0	0	18
	Botrytis	6	23	0	32	40	22	19	46	30	6	31	80	13	8	19	26	19	16	25
Relative mean signal		0 10 20 30 40 >50																		
Pectin																				

Arabidopsis CW modification during *Botrytis* infection

- Induction of linkage between HG, RG and hemicelluloses
- Addition of galactan side chains to RGI
- Xyloglucan galactosylation
- Callose deposition
- Induction of extensins and arabinogalactan proteins
- Modification of pectin methylesterification

Lionetti V. et al 2017 Plant Physiology 173:1–20

Lionetti V. and Metraux J. 2014. Frontiers in Plant Science. E-book ISSN 1664-8714 ISBN 978-2-88919-442-1

COLLECTION OF OLIVE STEMS FROM CULTIVARS WITH DIFFERENT RESISTANCE TO *Xylella*



RESISTANT

FS17

SUSCEPTIBLE

ARBEQUINA

Experimental site: plot in the epidemic area
Cutrofiano (Apulia, Italy)

*Stems were screened for *Xylella fastidiosa* presence*



Harper et al., 2010. Phytopathology 100(12):1282-8

Selected samples



The presence of *Xf* was not revealed



Xf was found in stems analyzed



For further information regarding the unpublished results, please contact
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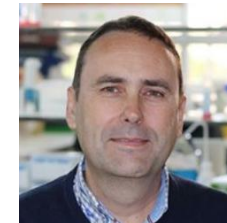


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XyWall **P R N**





Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026



REGIONE PUGLIA

INTEGRATED METAGENOMICS, METABARCODING AND CULTUROMICS REVEAL BENEFICIAL BACTERIA IN THE XYLEM MICROBIOME OF THE RESISTANT OLIVE CULTIVAR LECCINO

Di Cosmo D.¹, Touati R.², Del Grosso C.³, Franco M. E. E.¹, Giampetruzzi A.³, Donno R.¹, Masiello M.², Dugo Bati M.¹, Somma S.², Cozzi G.², Saldarelli P.³, Potere O.¹, Moretti A.², Faretra F.¹, De Miccolis Angelini R. M.¹, Saponari M.³, De Bellis P.²

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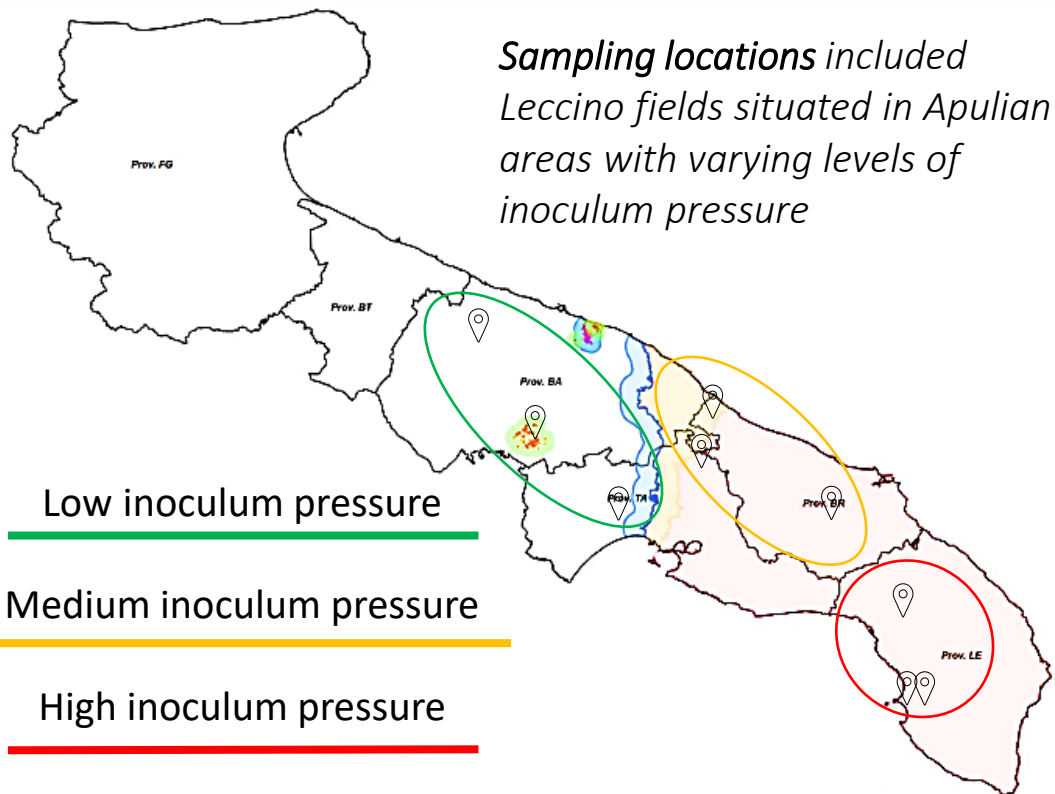
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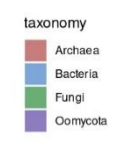
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MICROBIAL DETERMINANTS OF LECCINO RESILIENCE

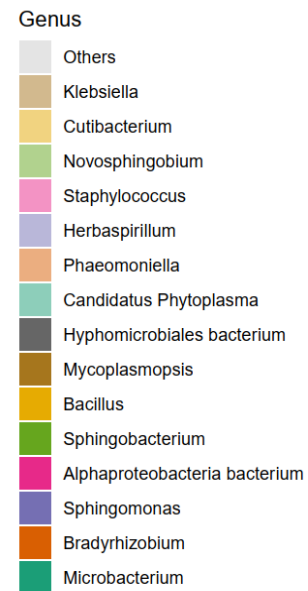
The **microbiome** associated with the resistant olive cultivar Leccino has been previously described, revealing bacterial taxa potentially linked to resilience against *Xylella fastidiosa* subsp. *pauca*. However, the ecological functions, genomic features and cultivability of these microbial communities remain largely unexplored.



Metagenomics, metabarcoding, and culturomics approaches were combined to characterize the Leccino xylem microbiome and to develop microbial resources for microbiome-based disease management. This study utilized 270 trees sampled from nine orchards differing in *Xylella fastidiosa* infection pressure.



Metagenomics revealed a **stable core microbiome** across locations and seasons, dominated by *Microbacterium*, *Sphingomonas*, and *Bradyrhizobium*.



Most abundant genera across locations detected in xylem wood by metagenomic analysis

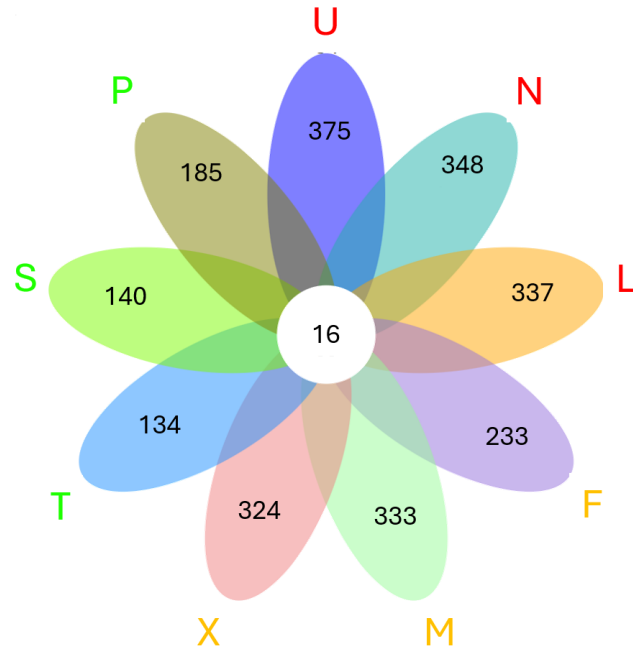
Number of shared genera across locations detected in xylem wood by metagenomic analysis

Region	Type	From	To	Similarity Confidence	Most similar known cluster
Region 13.1	T3PKS ↗	1,067	42,161		
Region 27.1	lanthipeptide-class-iii ↗	798	13,700		
Region 30.1	terpene-precursor ↗	86,109	107,170		
Region 45.1	RIPP-like ↗	21,909	32,556		
Region 58.1	NAPAA ↗	3,131	37,069	High	ε-Poly-L-lysine ↗ NRPS:Type
Region 76.1	terpene ↗	69,048	93,188	Low	carotenoid ↗ terpene
Region 77.1	betalactone ↗ , terpene-precursor ↗	1	26,636		

Predicted gene clusters for secondary metabolites in Microbacterium MAG

Functional analyses identified traits associated with **microbial competition**, including siderophore and secondary metabolite biosynthesis, quorum sensing and biofilm formation; MAGs were reconstructed for downstream **genome mining**.



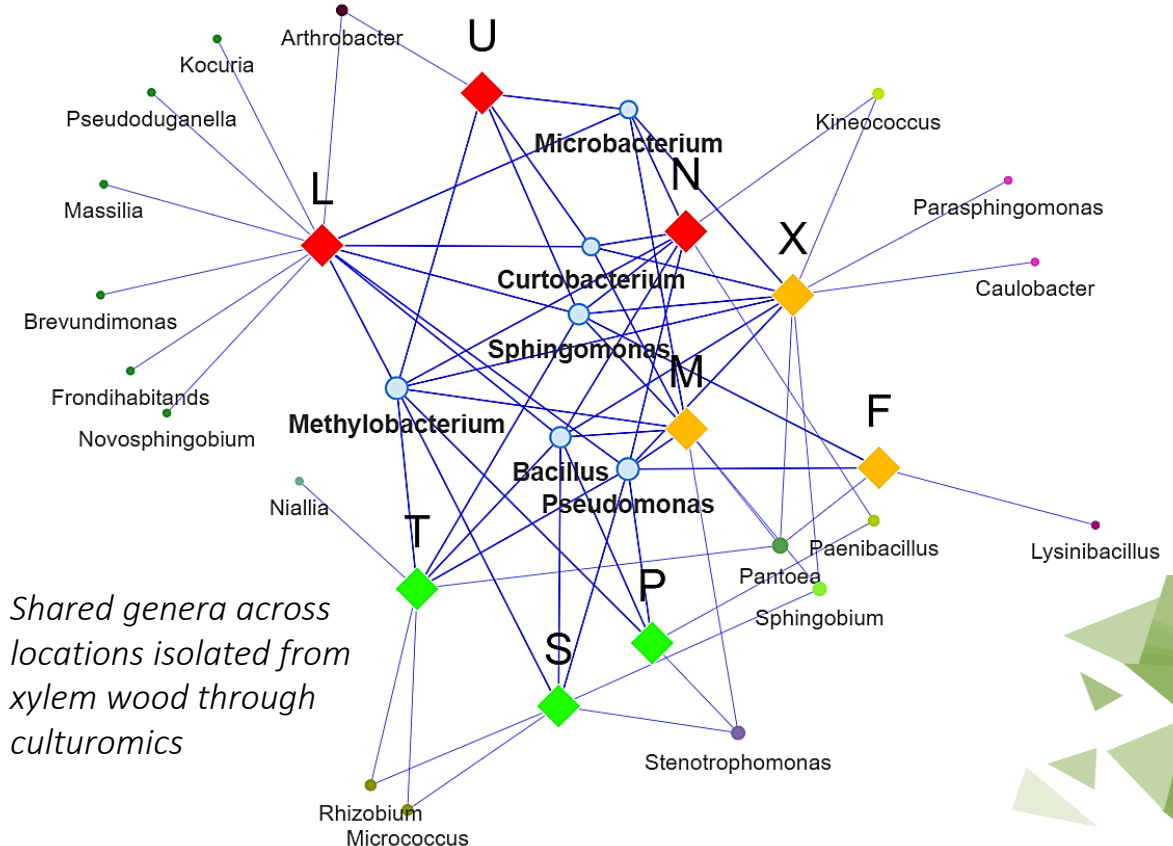


Number of shared genera across locations detected in xylem sap by metabarcoding analysis

Culturomics yielded 500 bacterial endophytic isolates belonging to 96 species and 38 genera, establishing a direct link between sequence-based observations and cultivable strains.

IDENTIFYING AND RECOVERING CANDIDATE TAXA

Metabarcoding identified 16 bacterial genera consistently associated with xylem sap across locations, including taxa with reported plant-beneficial and antagonistic traits.



CONCLUSIONS

The integration of metagenomics, metabarcoding and culturomics enabled characterization of the Leccino microbiome from community to isolate level.

Convergent taxonomic, functional and cultivation-based evidence identified candidate microorganisms and traits potentially associated with Leccino resilience.

MAGs and cultivable isolates constitute complementary resources for functional validation.

These results provide a framework for the rational design of synthetic microbial communities for the biological control of *Xylella fastidiosa*.



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