2nd European conference on Xylella fastidiosa | 2019

Insights into differential responses of olive cultivars to *Xylella fastidiosa* infections

(a roundtrip from citrus/grape to olive)

PASQUALE SALDARELLI

CNR, Istituto per la Protezione sostenibile delle piante

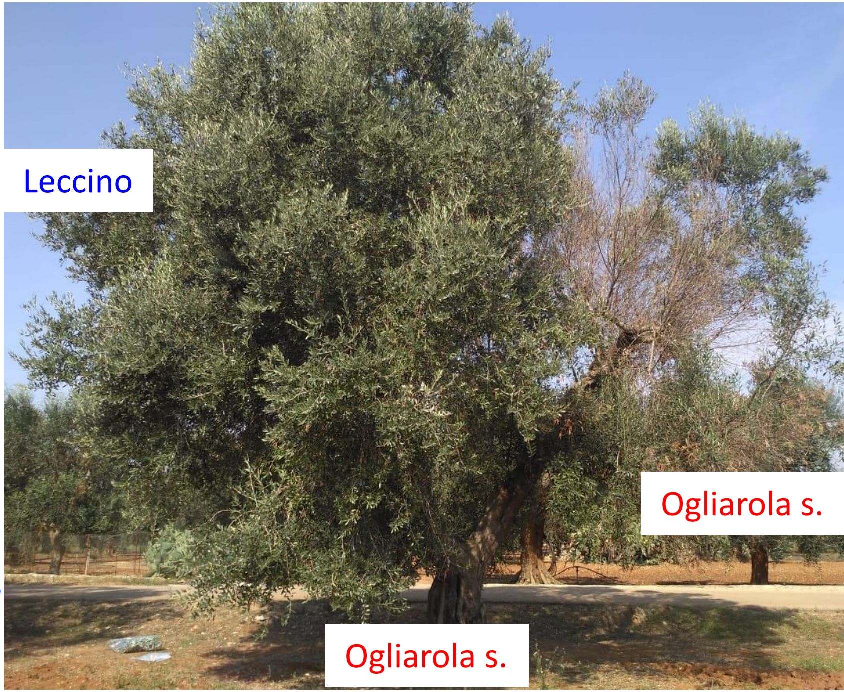
Bari, Italy

IPSP Istituto per la Protezione Sostenibile delle Piante
Consiglio Nazionale delle Ricerche

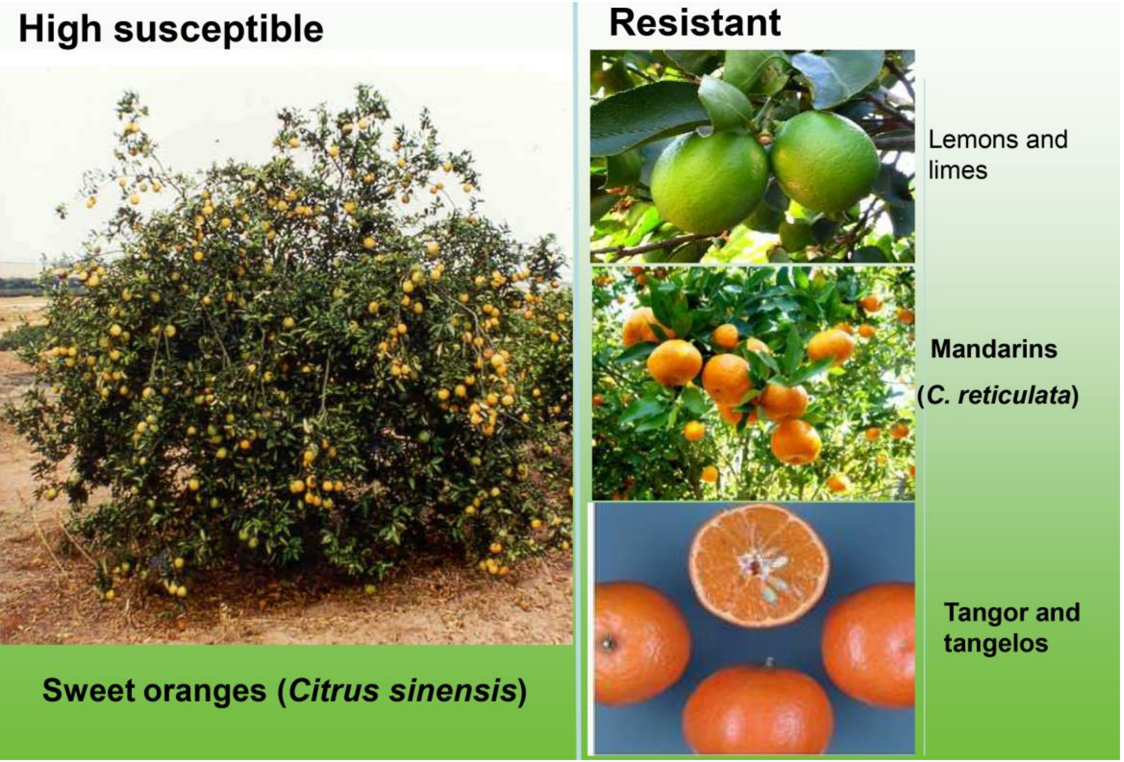
European Commission
Horizon 2020
European Union funding for Research & Innovation

POnTE Pest Organisms Threatening Europe

XF ACTORS
Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy



Xylella resistance in citrus (citrus variegated chlorosis)



RESEARCH ARTICLE Open Access

RNA-Seq analysis of *Citrus reticulata* in the early stages of *Xylella fastidiosa* infection reveals auxin-related genes as a defense response

Carolina M Rodrigues[1], Alessandra A de Souza[1], Marco A Takita[1], Luciano T Kishi[2] and Marcos A Machado[1*]

Up-regulated

Leucine-rich repeat receptor-like kinase (LRR-RLK) At4G08850, At3G47570

Differential colonization patterns of Xylella fastidiosa infecting citrus genotypes

B. Niza[ab], H. D. Coletta-Filho[a], M. V. Merfa[ab], M. A. Takita[a] and A. A. de Souza[a*]

- Colonization impaired in resistant
- No different xylem morphology
- Lignin deposition

Brasile

credits A. De Souza

Xylella resistance in *Vitis*, *Walker*, 2017

- *V. arizonica/candicans* b43-17 has single dominant gene for resistance to PD and it's homozygous
- All progeny from crosses to b43-17 are resistant to PD
- Genetically mapped PD resistance (*PdR1*), to chromosome 14. Linked markers have been used for marker-assisted selection (MAS)

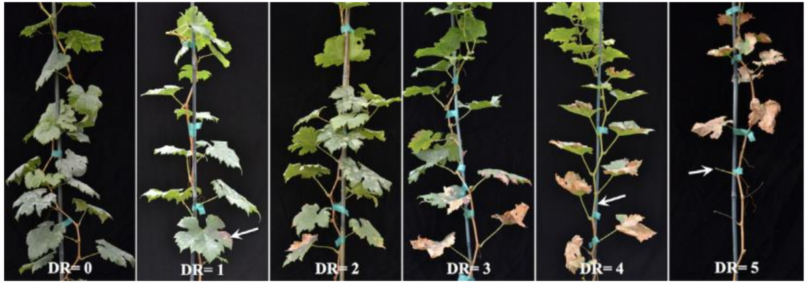
Controlling Pierce's Disease with Molecular and Classical Breeding

M. Andrew Walker

Features of *Xylella resistance* in *Vitis*, 2019

Assessment of Pierce's disease susceptibility in Vitis vinifera cultivars with different pedigrees

E. Deyett[a†], J. Pouzoulet[b†], J.-I. Yang[c], V. E. Ashworth[a], C. Castro[d], M. C. Roper[d] and P. E. Rolshausen[a*]



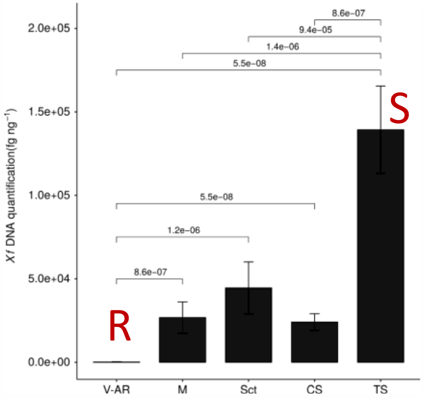
Disease severity

Genetic

In b43-17 V.arizonica/candicans PdR1 locus contains candidate Leucine-rich repeat receptor-like kinase receptors

LRR-RLKs

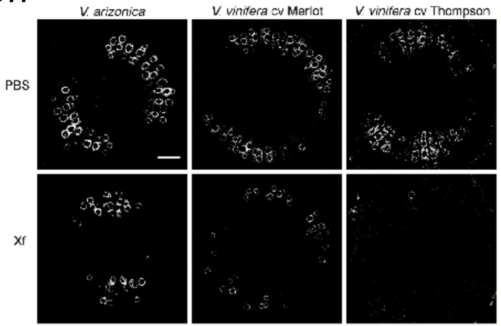
Pathogen abundance



Smaller vessel diameters=resistance

% of vascular occlusion

Anatomic



Features of Xylella resistance in *Vitis,* 2011

Assessment of the Process of Movement of *Xylella fastidiosa* Within Susceptible and Resistant Grape Cultivars

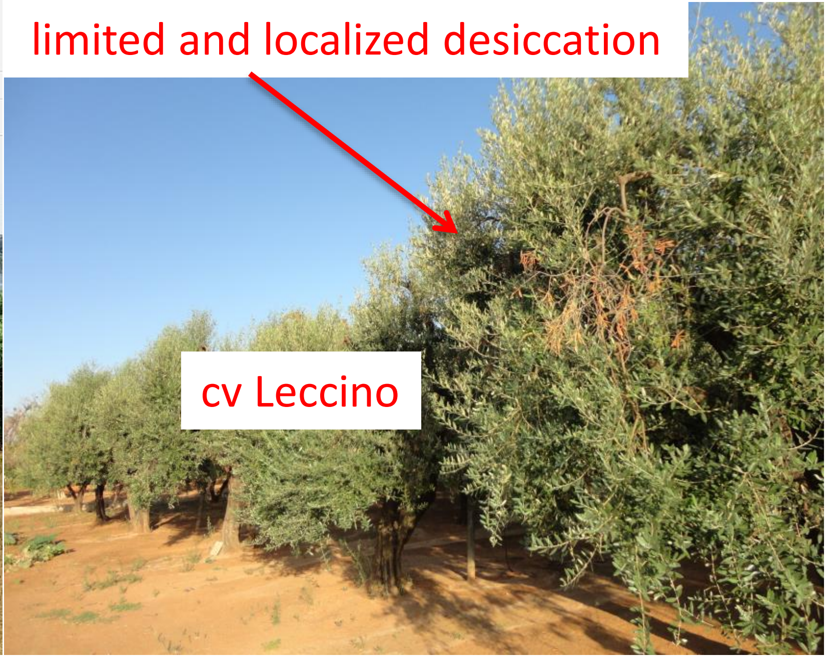
C. Baccari and S. E. Lindow

STEM

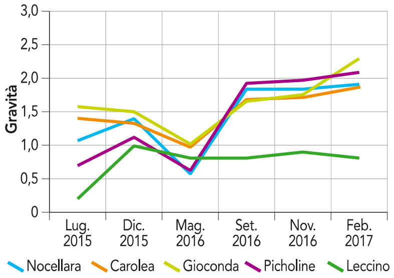
- Lower population sizes in resistant cv compared to susceptible

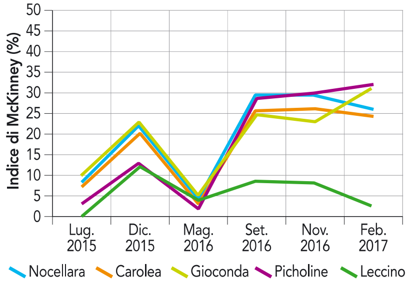
- Few vessel colonized in resistant cv compared to susceptible

Features of Xylella resistance in olive



Disease severity

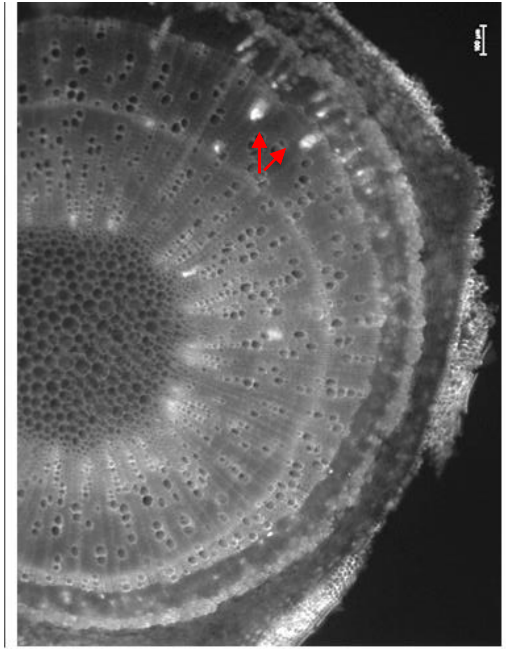


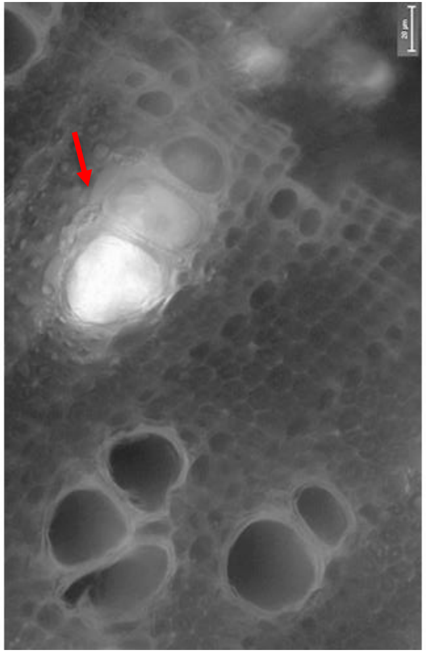


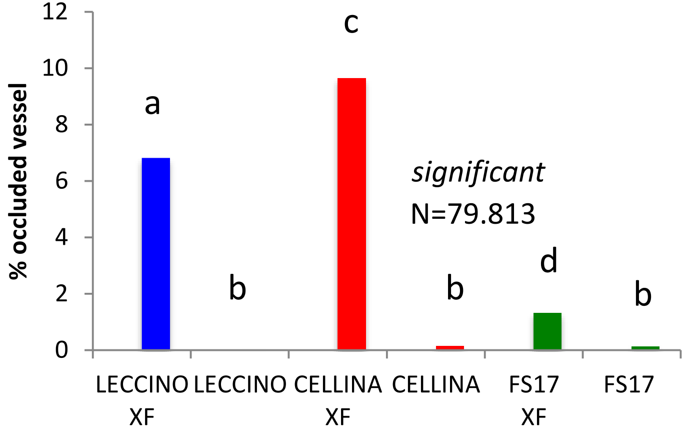
Boscia et al., 2017

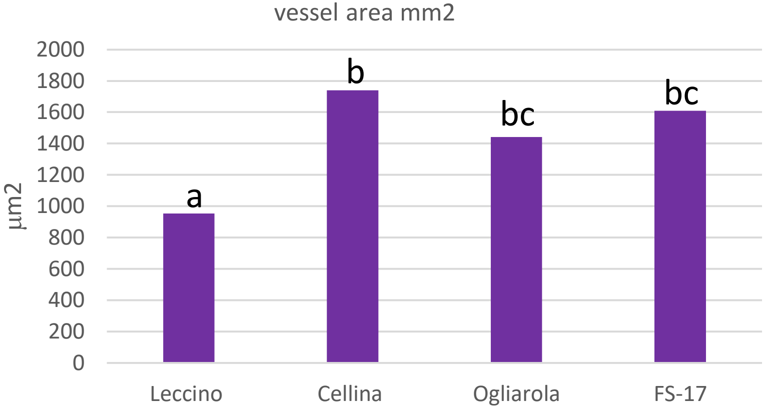
Features of Xylella resistance in olive

Anatomic









significant p<0.5

Features of Xylella resistance in olive

Pathogen abundance

TABELLA 2 - Risultati dell'analisi molecolare su piante di FS-17®, Kalamata, Leccino e Ogliarola salentina

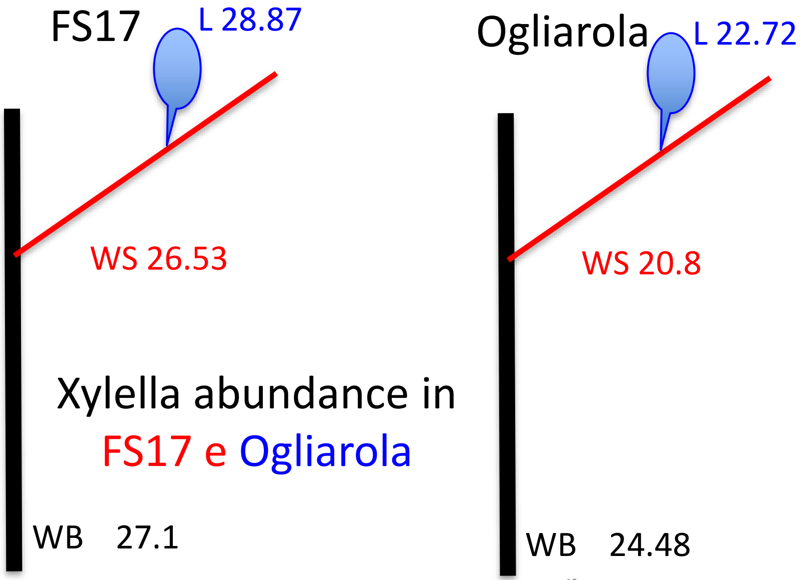
Cultivar	Positivi/Totale	Cq media positiva	Assorbanza media positivi
FS-17®	18/51	29,59 cicli	$5{,}04 \cdot 10^4$ UFC/mL
Kalamata	28/51	27,21 cicli	$3{,}16 \cdot 10^5$ UFC/mL
Leccino	9/18	28,41 cicli	$9{,}93 \cdot 10^4$ UFC/mL
Ogliarola salentina	10/10	26,41 cicli	$4{,}51 \cdot 10^6$ UFC/mL

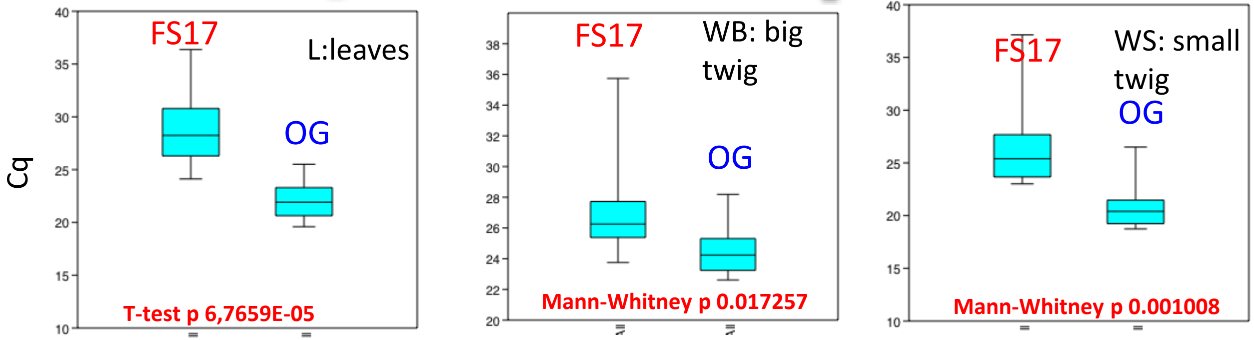
Features of Xylella resistance in olive

Pathogen abundance

10 plants each

Lower abundance in Leaves/petioles



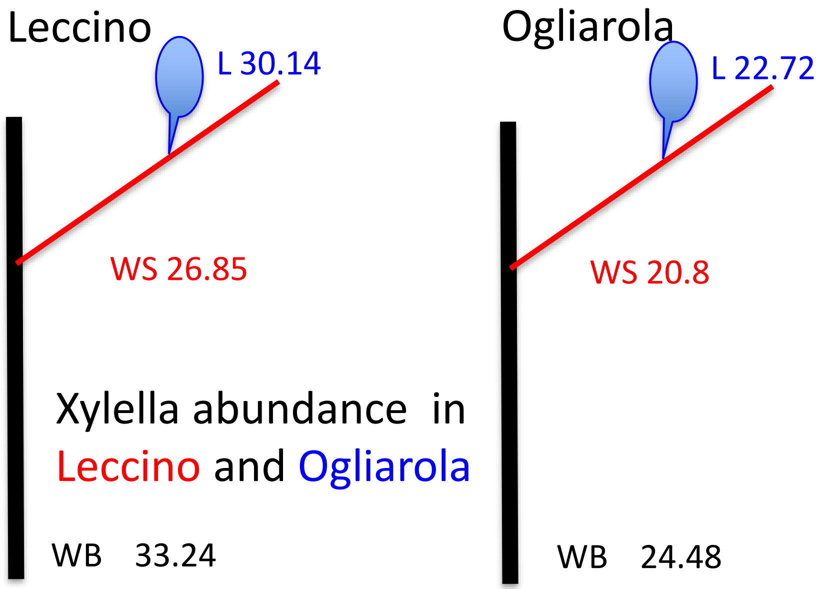


Features of Xylella resistance in olive

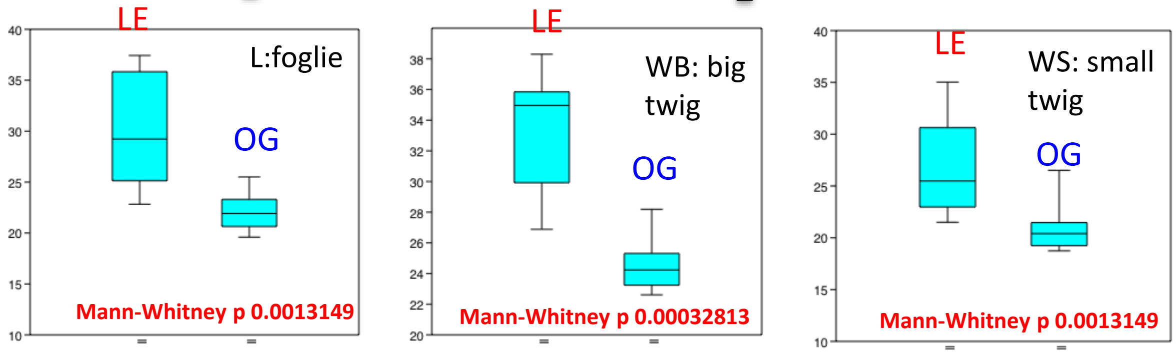
Pathogen abundance

10 plants each

Lower abundance in
Leaves/petioles
And WB

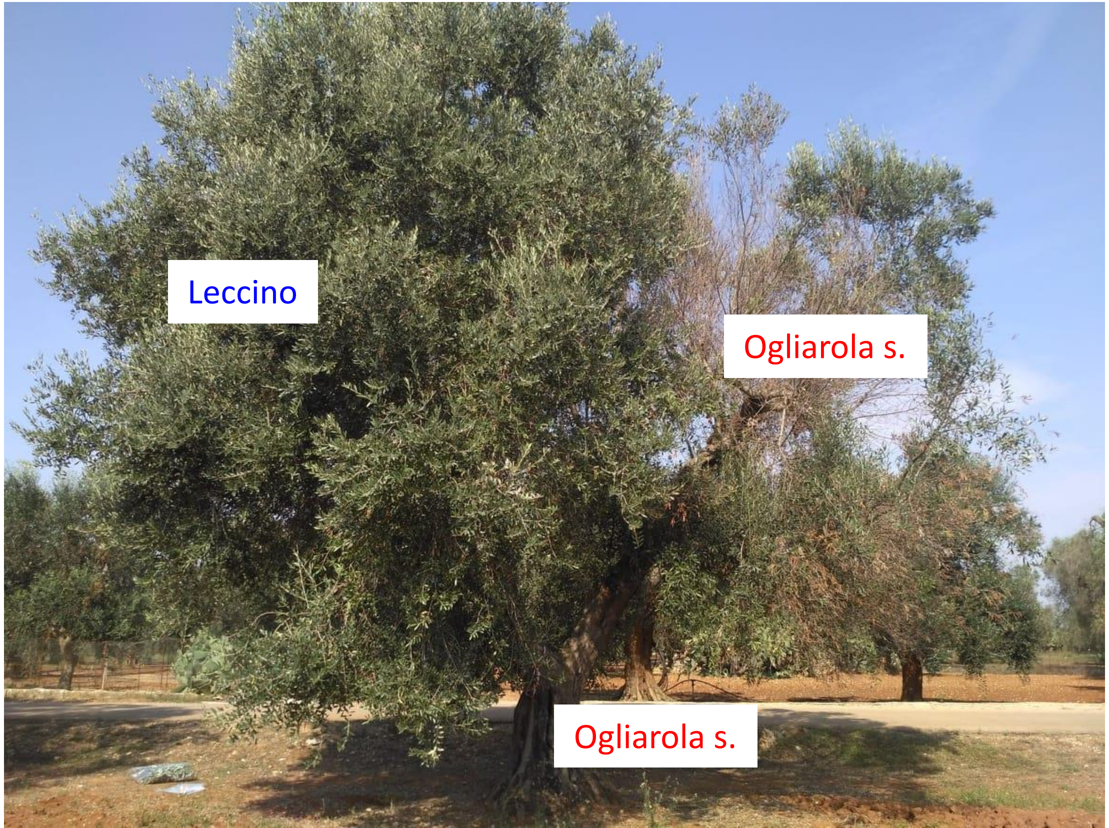


Xylella abundance in
Leccino and Ogliarola



Leccino resistance: further evidences

Leccino grafted on Ogliarola in the past
3 plants



Xf
CFU/ml

Leccino1
$1x10^4$
Ogliarola1
$8x10^5$

Leccino2
$5x10^2$
Ogliarola2
$1x10^6$

Leccino3 N/D
Ogliarola3
$9x10^5$

Features of Xylella resistance in olive

Genetic

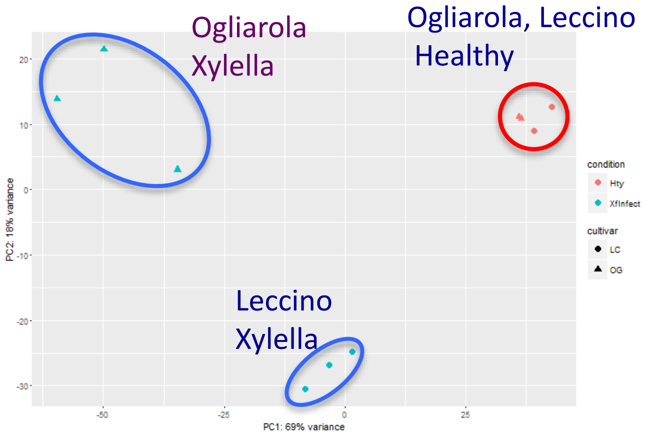
Giampetruzzi *et al. BMC Genomics* (2016) 17:475
DOI 10.1186/s12864-016-2833-9

BMC Genomics

RESEARCH ARTICLE — Open Access

Transcriptome profiling of two olive cultivars in response to infection by the CoDiRO strain of *Xylella fastidiosa* subsp. *pauca*

Annalisa Giampetruzzi[1], Massimiliano Morelli[2], Maria Saponari[2], Giuliana Loconsole[1], Michela Chiumenti[2], Donato Boscia[2], Vito N. Savino[1], Giovanni P. Martelli[1] and Pasquale Saldarelli[2*]



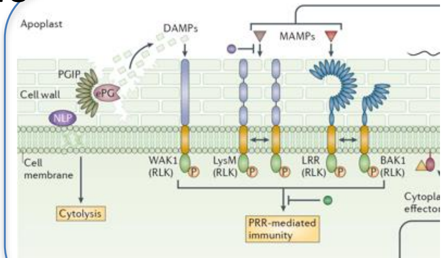
Ogliarola

↑ expression

- *early light-induced proteins (ELIP)*
- *Late embryogenesis abundant protein (LEA)*
- Expansin- like B1 (osmoprotectant)
- ABA2 (abscisic acid)
- deidrina (LEA protein)

Ogliarola suffers a drought stress upon Xf infection

Leccino



Wirthmueller L et al. 2013.

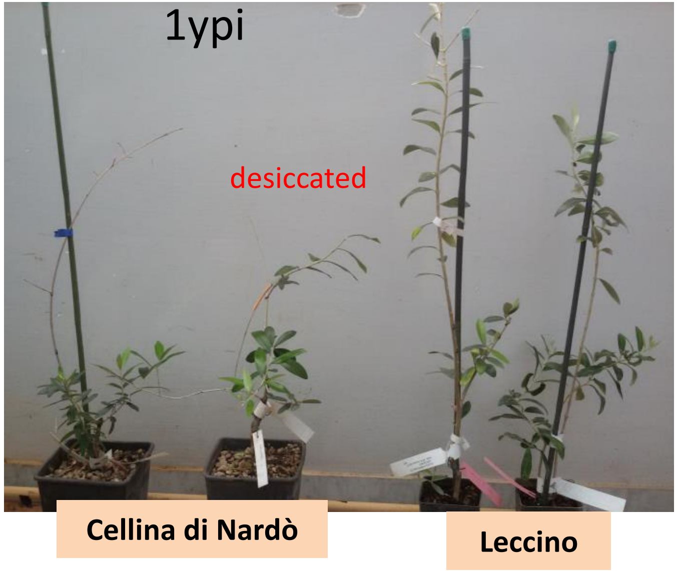
Several *LRR-RLKs*
LRR-RLK - At1g35710

↑ expression

LRR-RLK: Leucine-rich repeat receptor-like kinases

Transcriptome studies of greenhouse trials

Xf strain	trial		
CO33	3x Cellina	3x Leccino	2x Mock
De Donno	3x Frantoio		2x Mock
De Donno	3x Fs17		2x Mock



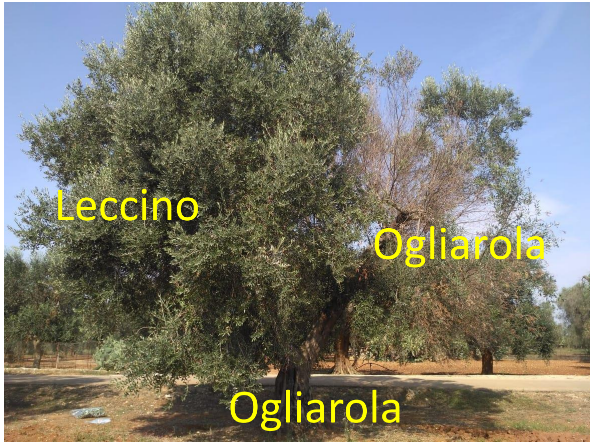
Late (cronic) infections

Features of Xylella resistance in olive

Genetic

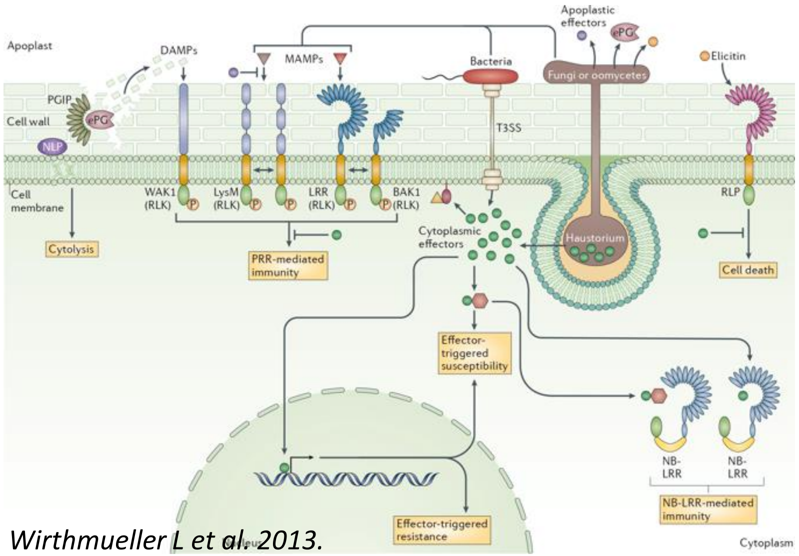
Transcriptome studies of different field trials

Xf strain	trial		
De Donno	3x Leccino	3x Ogliarola	4x Mock
De Donno	3x Ogliarola grafted Leccino		



Late (cronic) infections

Summary of transcriptome studies



Wirthmueller L et al. 2013.

Common traits

Leucine-rich repeat receptor-like kinase receptors

LRR-RLKs UP-Regulated

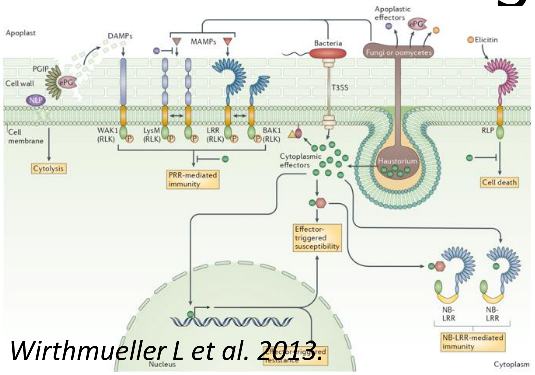
- *At1g35710*
- *At3g47570*
- *At4g08850*

Features of Xylella resistance in olive

Genetic

Summary of transcriptome studies

common traits



Wirthmueller L et al. 2013.

Leucine-rich repeat receptor-like kinase *receptors*

LRR-RLKs UP-Regulated

At1g35710, At3g47570, At4g08850

LRR XII

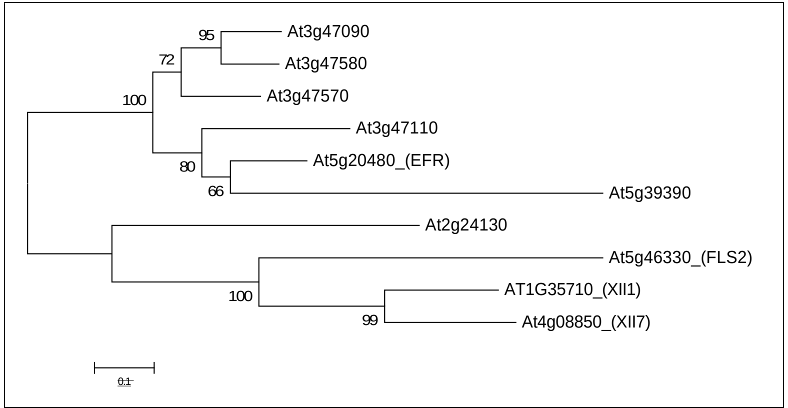


Figure 5-2 A Phylogenetic tree of LRR-RLK subfamily XII in *A. thaliana*

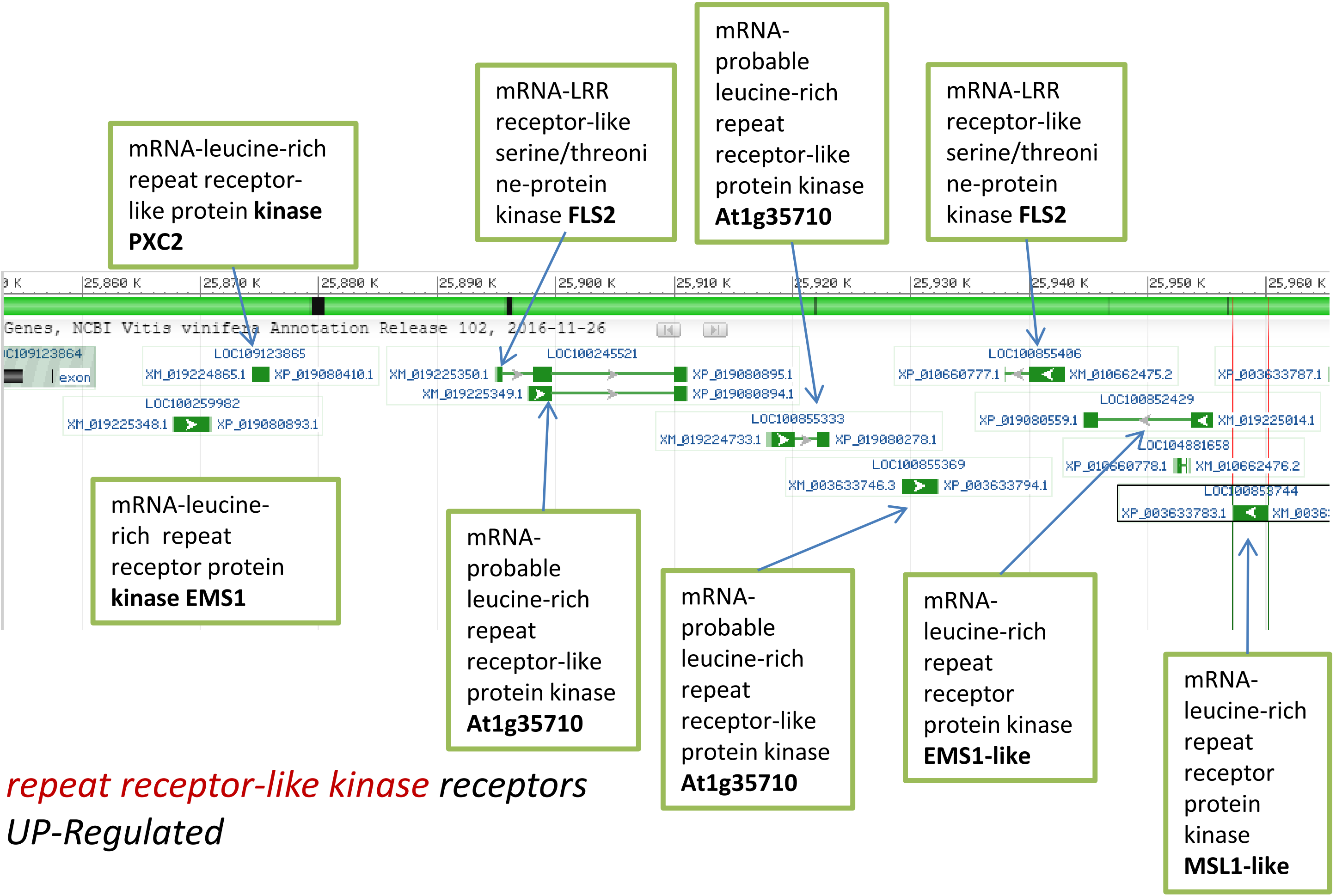
similar to well characterized
LRR-RLKs recognizing bacteria components

FLS2: Flagellin sensing 2

EFR: EF-Tu Receptor

McKenna JF thesis: *Analysis of the signaling mechanisms involved in Cell Wall Integrity maintainance in A. thaliana*

Vitis Vinifera NCBI Annotation, Pierce disease resistance locus (PdR1)



Leucine-rich repeat receptor-like kinase receptors

LRR-RLKs UP-Regulated

At1g35710

Citrus (early infection)

Rodrigues *et al. BMC Genomics* 2013, 14:676 Page 4 of 13
http://www.biomedcentral.com/1471-2164/14/676

Table 1 Differentially expressed genes in Ponkan mandarin in response to infection by *X. fastidiosa*

Gene symbol	gene id_*Citrus clementina**	AGI**	Fold change***	P ≤ 0.001	Gene description
LRR-RLK	Ciclev10004108m	AT4G08850	1.05611	0.000755101	leucine-rich repeat family protein
LRR-RLK	Ciclev10014130m	AT3G47570	2.64522	3.84525e-09	leucine-rich repeat protein kinase family protein
RLP12	Ciclev10008540m	AT1G71400	1.20144	2.69007e-06	receptor like protein 12
CC-NBS-LRR	Ciclev10007304m	AT4G27190	1.29361	4.28293e-06	nb-arc domain-containing disease resistance protein

Leucine-rich repeat receptor-like kinase receptors

LRR-RLKs UP-Regulated

At3g47570, At4g08850

PLOS | GENETICS

RESEARCH ARTICLE

The Arabidopsis leucine-rich repeat receptor kinase MIK2/LRR-KISS connects cell wall integrity sensing, root growth and response to abiotic and biotic stresses

Dieuwertje Van der Does[1], Freddy Boutrot[1], Timo Engelsdorf[2], Jack Rhodes[1], Joseph F. McKenna[3¤a], Samantha Vernhettes[4], Iko Koevoets[5], Nico Tintor[6], Manikandan Veerabagu[2], Eva Miedes[7], Cécile Segonzac[1¤b], Milena Roux[1¤c], Alice S. Breda[8], Christian S. Hardtke[8], Antonio Molina[7], Martijn Rep[6], Christa Testerink[5], Grégory Mouille[4], Herman Höfte[4], Thorsten Hamann[2,3], Cyril Zipfel[1]*

MIK2/LRR-KISS is a component of the cell-wall integrity (CWI) sensing

MIK2/LRR-KISS is a LRR-RLK encoded by **At4g08850**

MIK2 belong to the XIIb sub-family of LRR-RLKs and is homologous (60%aa identity) to **At1g35710**

Working Hypotesis

MIK2 (At4g08850 similar to At1g35710): regulator of cell wall damage responses, is a CWI sensor

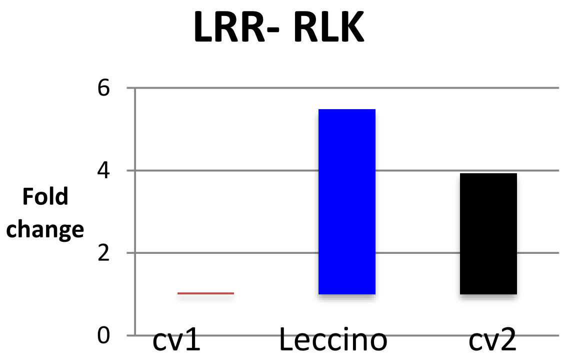
Xylella has a set of Cell Wall Degrading Enzymes (CWDE)

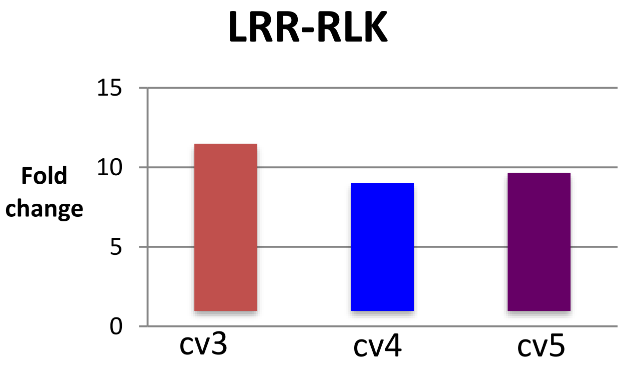
Disruption of Cell Wall Integrity >stress responses (ROS JA,SA, ET, lignin, callose, pect methyl-esterification)

Responses reminiscent of the Plant's Defence Reaction to pathogens and insects

MIK2 is required for resistance to the fungal root pathogen Fusarium oxysporum, which multiplies in the xylem and produce CWDE

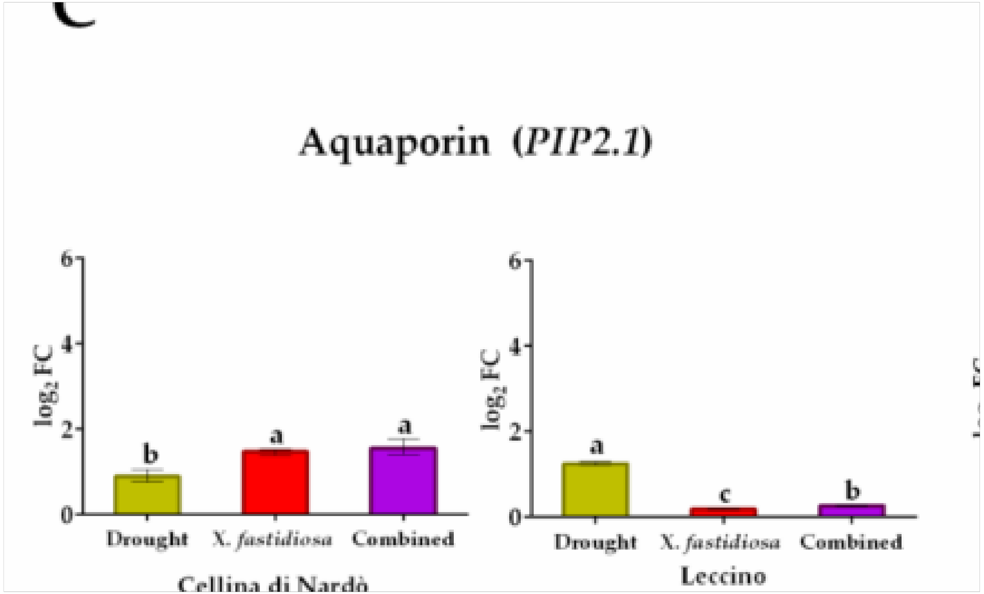
LRR-RLK detection in cultivars with diverse susceptibility/resistance to Xyella





greenhouse

ongoing



plants

MDPI

Article

Molecular Effects of *Xylella fastidiosa* and Drought Combined Stress in Olive Trees

Mariarosaria De Pascali [1], Marzia Vergine [1,*], Erika Sabella [1], Alessio Aprile [1], Eliana Nutricati [1], Francesca Nicolì [1], Ilaria Buja [2], Carmine Negro [1], Antonio Miceli [1], Patrizia Rampino [1], Luigi De Bellis [1] and Andrea Luvisi [1]

Why do we say “resistance”

SUSCEPTIBLE: *unable to resist to the infection of a specific pathogen*

IMMUNE: *does not allow infection by a specific pathogen*

TOLERANT: *able to contain the effects of an infection regardless of the pathogen replication*

RESISTANT: *able to limit the pathogen replication*

Pagan & Garcia-Arenal. Int. J. Mol. Sci. 2018, 19, 810; doi:10.3390/ijms19030810
Roy, B.A.; Kirchner, J.W.. Evolution 2000, 54, 51–63.
Agrios.2005. Plant pathology, Fifth Edition,

Conclusions

Strong analogies with resistance in grape (citrus)

- Pathogen abundance
- Percentage of occluded vessels
- Area of xylem vessels
- LRR-RLK involvment

ongoing

- ➢ Acquiring information on olive gene locus involved
- ➢ Xylella degrades cell-wall
- ➢ Degradation is sensed by specific LRR-RLKs (cell-wall integrity sensing)

PONTE
Pest Organisms Threatening Europe

European Commission
Horizon 2020
European Union funding
for Research & Innovation

XF ACTORS
Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy

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Donato Boscia
Maria Saponari

2nd European conference on Xylella fastidiosa | 2019

IPSP
Istituto per la Protezione Sostenibile delle Piante
Consiglio Nazionale delle Ricerche