

# European Research on *Xylella fastidiosa*

## H2020 funded projects

2015  
-  
2019



2016  
-  
2020

Xylella Fastidiosa Active Containment Through a  
multidisciplinary-Oriented Research Strategy

Maria SAPONARI

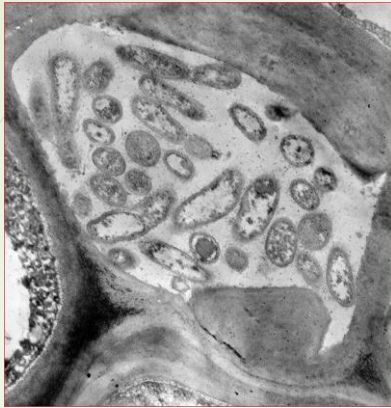
Institute for Sustainable Plant Protection, CNR Bari (Italy)





# *Xylella fastidiosa*

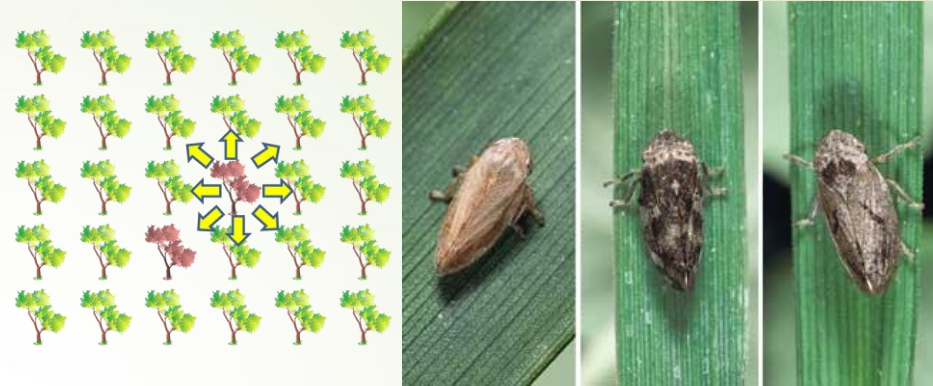
- Xylem-limited bacterium



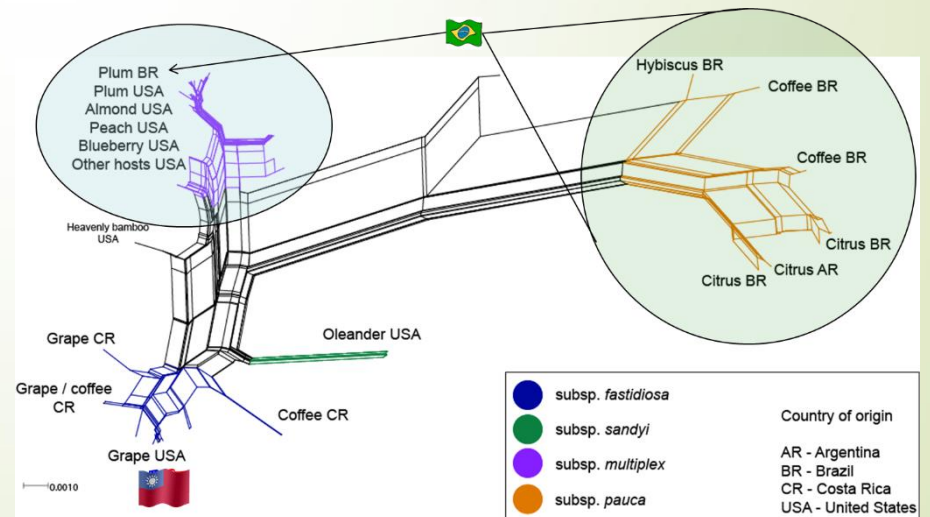
- >560 plant species
- Several severe diseases



- Insect vectored - xylem sap-feeding insects

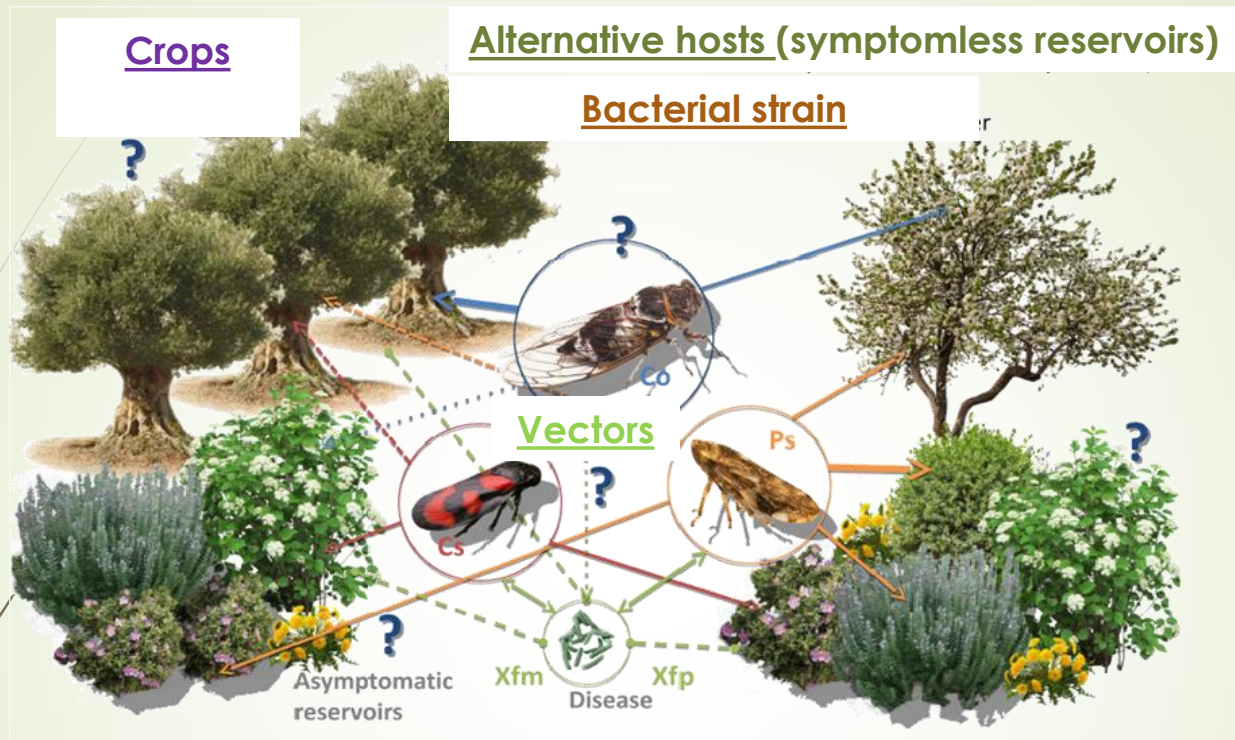


## Subspecies - geographic distribution – host specificity





# *X. fastidiosa* diseases have very COMPLEX biotic and abiotic interactions



- ✓ environment (e.g. temperature)
- ✓ vector ecology
- ✓ pathogen ecology
- ✓ host plant ecology
- ✓ outcome of various interactions
- ✓ disease management





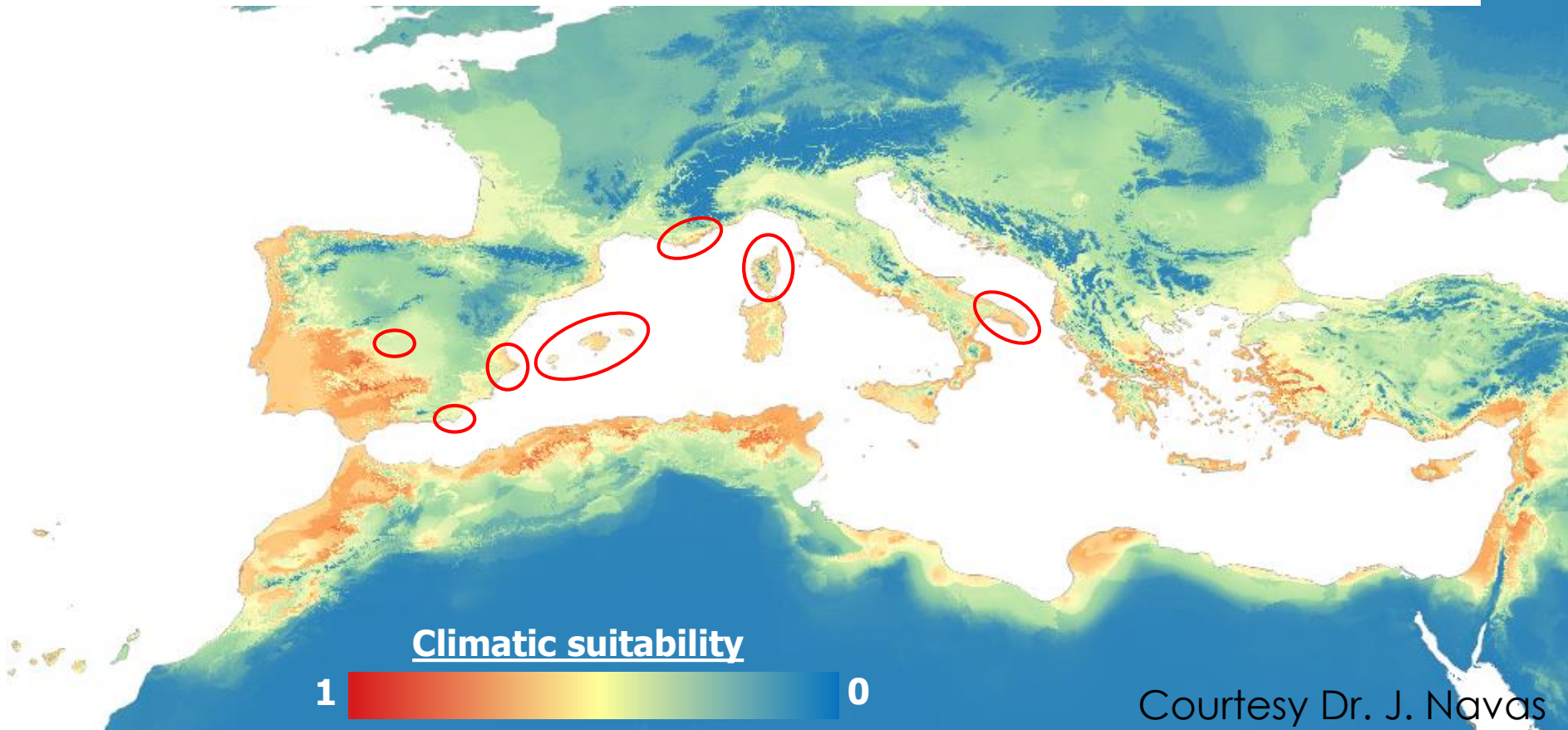
# Risk reduction options

- Prevention
- Surveillance (Early detection)
- Removal/Reductions sources of inoculum
- Vector controls
- Use Xf-free plants
- Thermotherapy (grapes)



## Current distribution of *Xylella* in EU

- Three countries dealing with outdoor outbreaks
- 77 host species, two major crops severely affected: olives and almond
- Strains of three different subspecies detected





**First identification of XF = consequence of the finding of a new severe olive disease**

**6**

## **Disease spread, symptom progression and severity**





Olive trees showing quick decline syndrome at advanced stage





# Gallipoli (Lecce), October 2013





# Gallipoli (Lecce), April 2016





# Gigante di Alliste (1500 anni)

30 Settembre 2014





July 2016





# Young trees

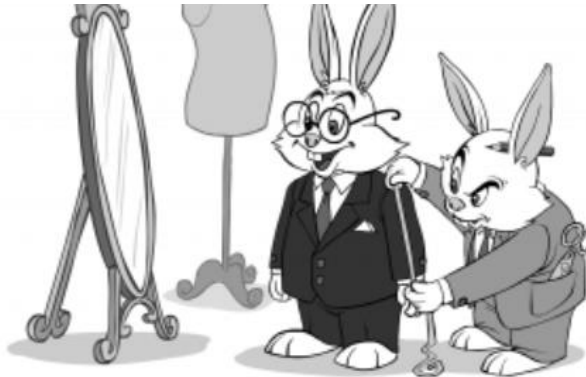




# One size doesn't fill all



ONE SIZE  
FITS ALL



MADE TO  
MEASURE

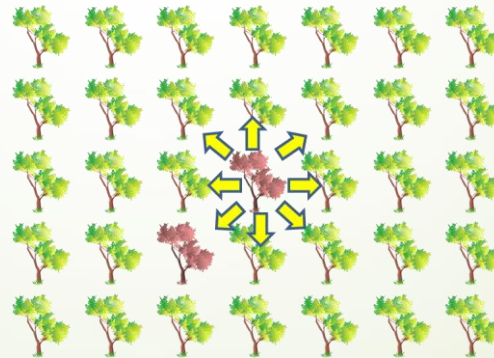
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**CONTROL**



**Vector(s)**



**Host(s)**

**Spread**



# Partnership

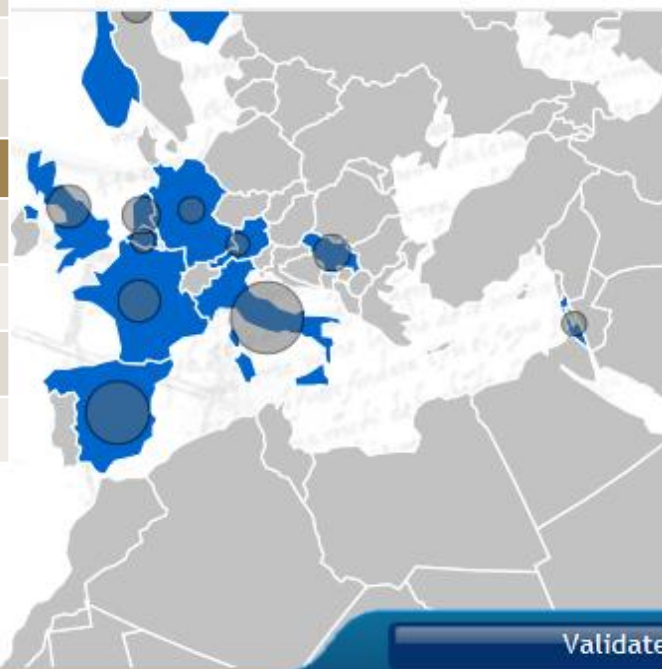
## EU COUNTRIES

ITALY	CNR-IPSP, UNIBA-DiSSPA , AGRITEST, ACLI RACALE
FRANCE	INRA, ANSES, VILMORIN
SPAIN	CSIC-ICA, CSIC-IAS, IVIA-AC, IVIA-PPBC, CITOLIVA, AGR VILLENA
BELGIUM	AUREA IMAGING
NETHERLANDS	WU, CERTIS EUR
AUSTRIA	BFW
UK	SG-SASA, FORESTRY RES AG, A L TOZER
GERMANY	LOEWE, PRC
FINLAND	LUKE

## NON-EU COUNTRIES

COSTA RICA	UCR
ISRAEL	ARO VOLCANI
SERBIA	UB-FA
NORWAY	NIBIO

Plant pathologist  
Bacteriologists  
Entomology  
Micologists  
Agricultural engineering  
Seed producers  
Associations of growers



**13 COUNTRIES – 25 PARTNERS**



# 14 COUNTRIES – 29 PARTNERS

## EU COUNTRIES

### ITALY

CNR-IPSP, UNIBA-DiSSPA , CREA,  
CEMCC, CIVI-Italia, Enbiotech Srl

### FRANCE

INRA, CIHEAM, EPPO

### SPAIN

CSIC, IVIA, IFAPA, AINIA

### BELGIUM

IVLO, JRC

### NETHERLANDS

NVWA, NAKTUINBOUW

### GREECE

BPI

### UK

NERC, USAL, RUSSEL IPM

### GERMANY

JKI

### SWEDEN

IFOAM

### PORTUGAL

IPB

## NON-EU COUNTRIES

### COSTA RICA

UCR

### BRAZIL

IAC

### TAIWAN

NTU

### USA

UC BERKELY





## **MAIN OBJECTIVES OF THE PROJECTS**

- **DEVELOP KNOWLEDGE**
- **ANSWER TO IMPORTANT EPIDEMIOLOGICAL QUESTIONS** (PATHWAY OF INTRODUCTIONS AND DISEASE SPREAD)
- **PRACTICAL SOLUTIONS AND MEANS FOR PREVENTION AND CONTROL**



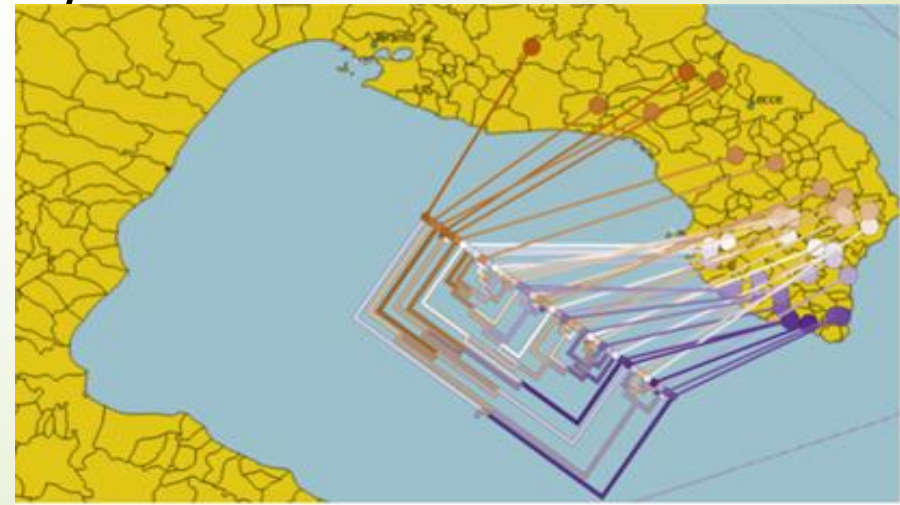
# BACTERIUM

**Determined its role (OQDS) and pathogenicity**

**Sequenced its full genome:**

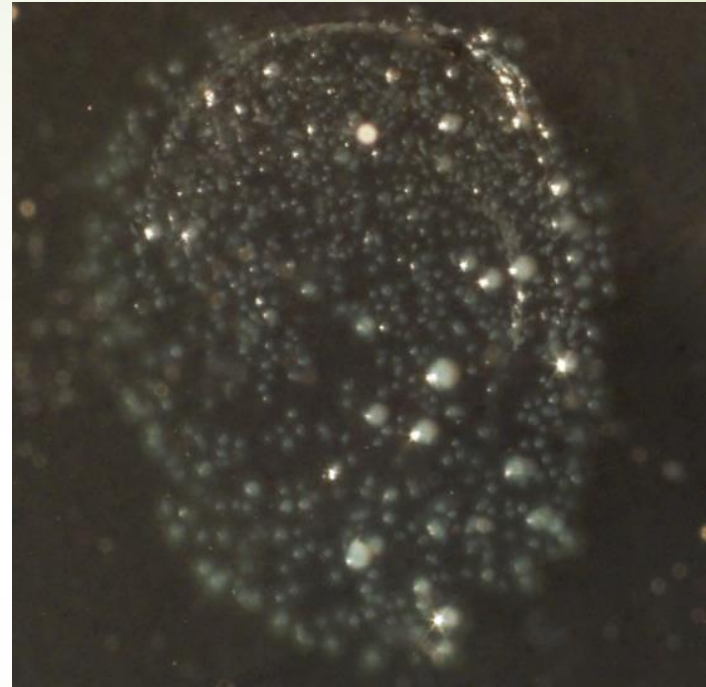
- clarified the pathway of introduction (central America)
- Possibility to understand how the bacterium is spreading (wave-jumps?)

**Implications for the control measures**





# Koch's postulates fulfilled





# Pathogenicity:

- PROTOCOL TO SCREEN CULTIVAR SUSCEPTIBILITY
- ASSESS THE IMMUNITY OF THE SPECIES («DE-LISTING» - MOVEMENTS OUTSIDE DEMARCATED AREA)





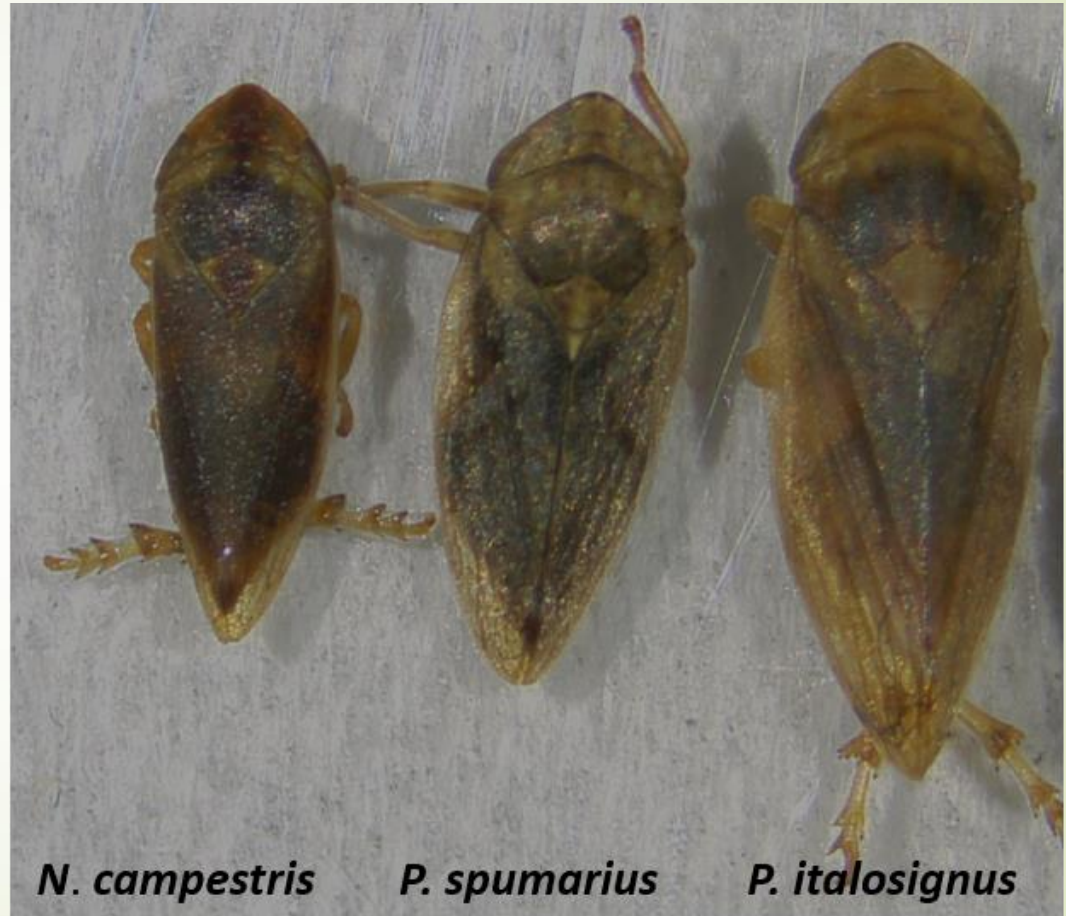
# Vectors: the role of spittlebugs

**2014-2017**

*Philaenus spumarius*

- Predominant species
- Major vector species

# ! Control !



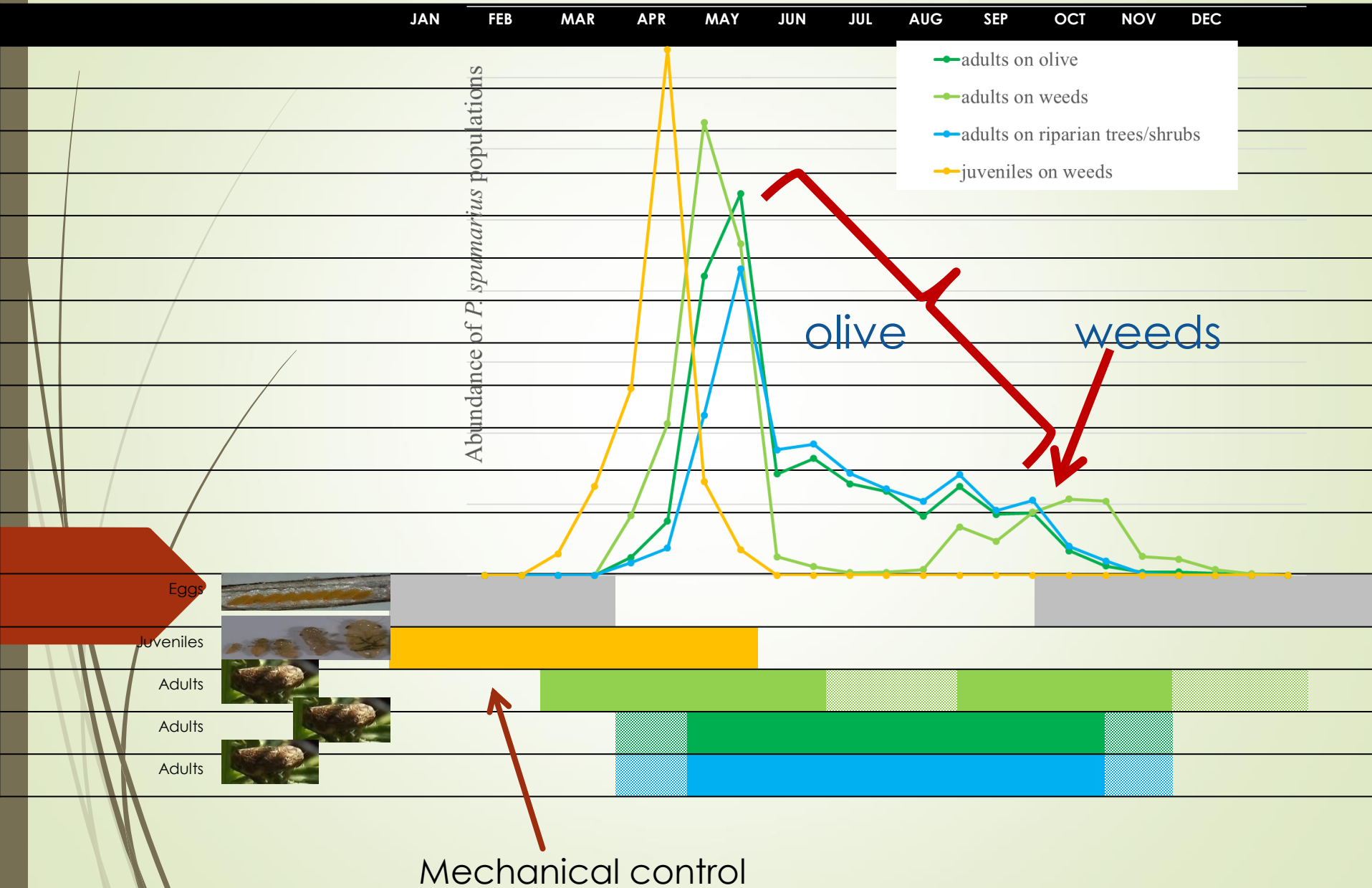
*N. campestris*

*P. spumarius*

*P. italosignus*



# Biological cycle of *P. spumarius* in Apulia





# HOSTS: OLIVES

## ➡ HOST RESISTANCE

FIELD EVIDENCE





**IS THIS SOMETHING CONSTITUTIVE OF THE VARIETY?**

**ARE THERE OTHER VARIETES RESISTANT OR TOLERANT?**

**IS THIS SPECIFIC FOR CERTAIN STRAIN-CULTIVAR COMBINATIONS?**





## Plants 1-year post-inoculation

Plants inoculated  
with a sandyi strain



**Cellina di Nardò**

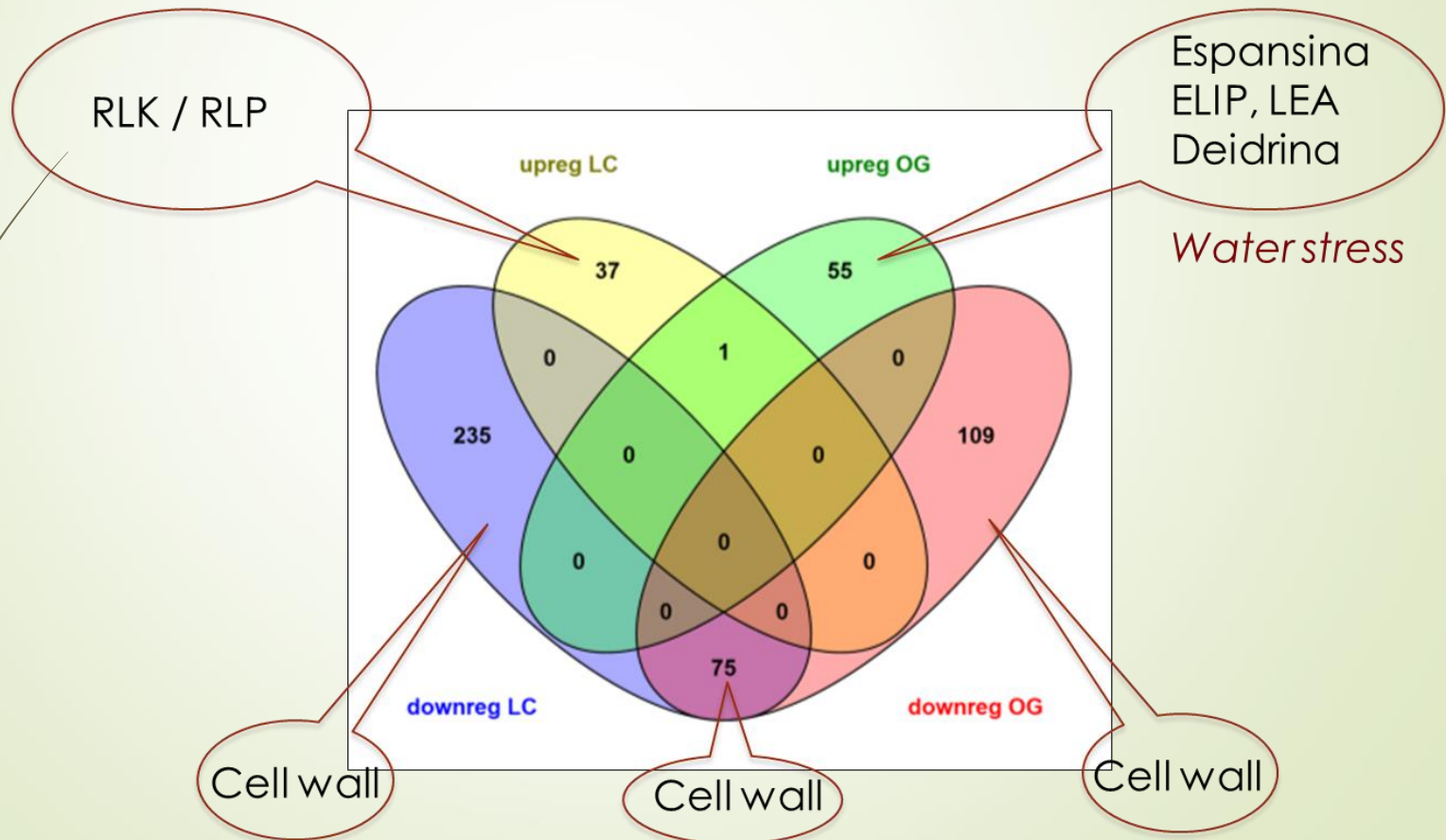
**Leccino**

Arrows indicate dessicated



# MOLECULAR STUDIES: TRANSCRIPTOMES

- 2 CULTIVARS IDENTIFIED WITH TRAITS OF RESISTANCE
- RESISTANCE= HARBOR LOWER BACTERIUM = LOWER VECTOR TRANSMISSIBILITY
- LECCINO UPON INFECTIONS ACTIVATES GENES AGAINST XF
- OGLIAROLA ACTIVATES WATER STRESS GENES





# These studies support the evidence that genetic resistance exists in olive

- Large program of screening for olive cultivars





## WORLD COLLECTION IN CORDOBA

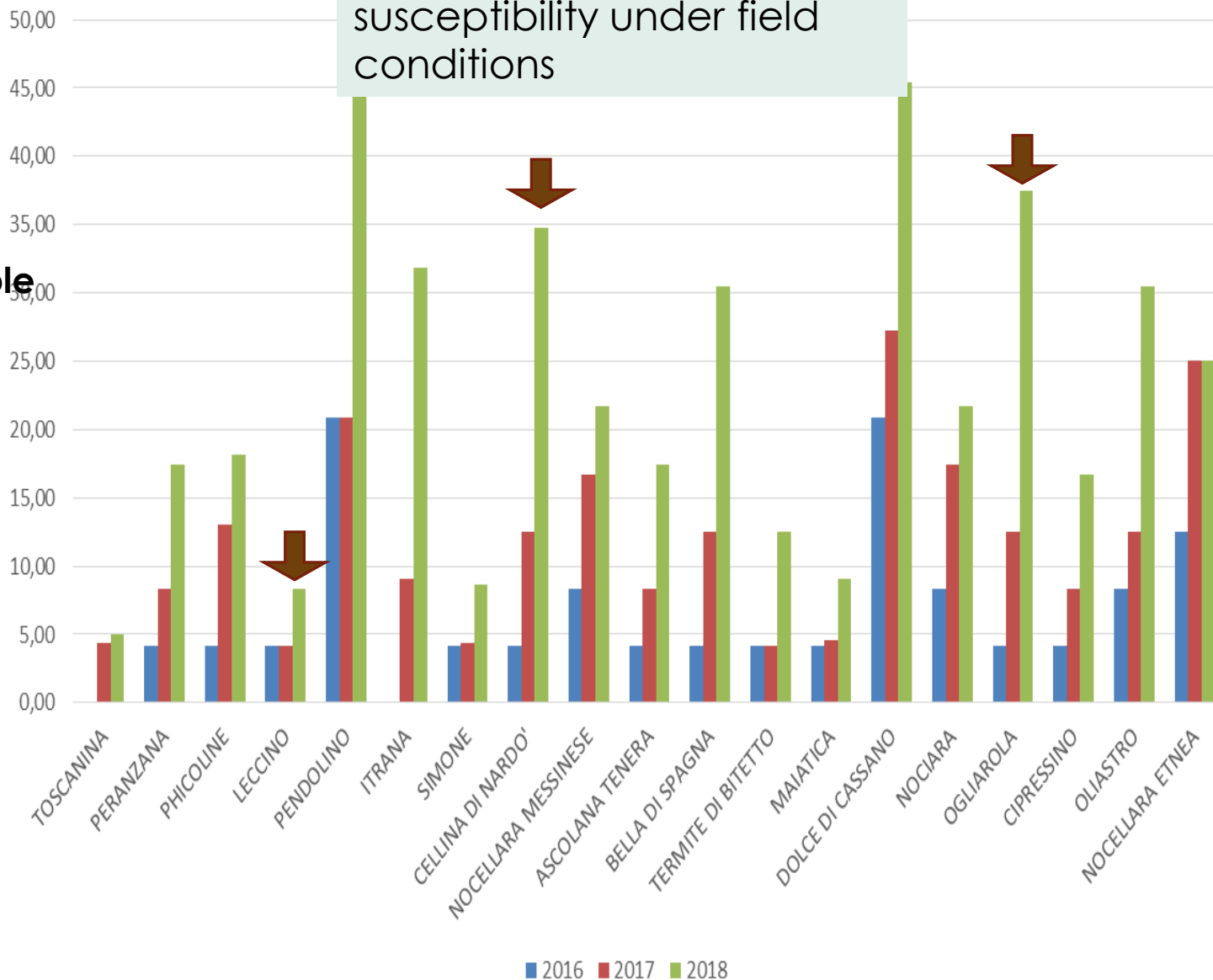
- 60 SELECTIONS SENT IN ITALY – SCREENING AGAINST THE PAUCA STRAIN
- FROM ALL OVER THE MEDITERRANEAN COUNTRIES





# Infection rates (%) on 19 olive cultivars exposed to natural infections from

Initial evidence of different susceptibility under field conditions



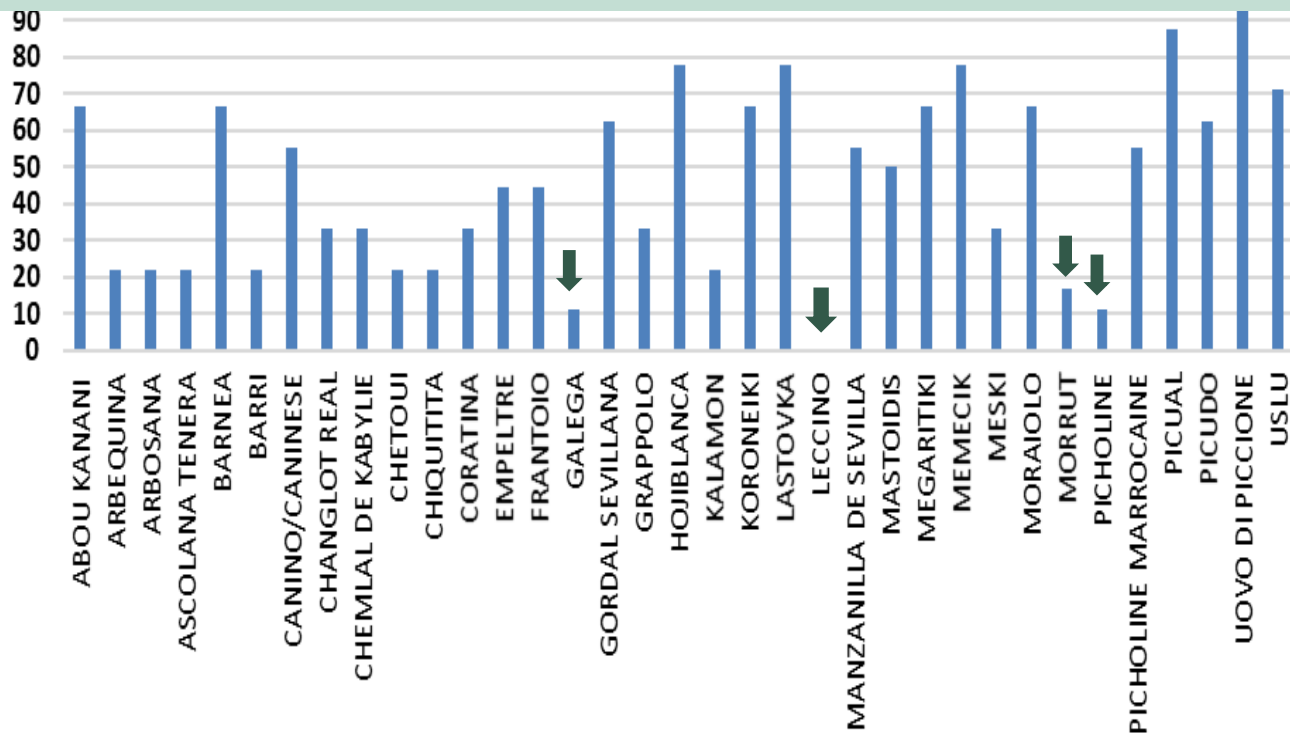


## Task 6.5 Artificial inoculations (started in 2017) (press)



Differences in the rate of plant colonization are detected, but longer period of observations and testing are necessary

**% of inoculated plants in which the bacterium could be detected at approx. 10cm above the points of inoculation after 6-months**





# SELECTION OF WILD GENOTYPES WITH PROMISING PHENOTYPES

- Search in the highly infected area of symptomless olive trees





# COMPLEMENTARY WORK IN SPAIN: SCREENING AGAINST MULTIPLEX STRAIN







**Alfafara  
Arbequina  
Frantoio  
Picual**

**Field plots in  
Ibiza and  
Mallorca**



# Alicante – olives Xf-free

STRAIN DIFFERENT FROM THE ITALIAN







# «CONSTRAINS AND HOPE»

- FACILITIES FOR SCREENING TESTS
- OLIVE GENOME (FARGA, ANNOTATION STILL UNDER IMPLEMENTATION)
- IDENTIFY GENETIC MARKERS FOR THE RESISTANCE
- PROVIDE TO GROWERS INFO ON RESISTANCE TO XF





# OTHER STUDIES FOR THE CONTROL OF THE PATHOGEN IN THE HOSTS

- MICROBIOME – ENDOPHYTES (are they involved in the resistance?)
  - **PARABURKHOLDERIA PHYTOFIRMANS**
- Antimicrobial PEPTIDES
- Bacteriophages (not successful yet)



# Characterization of the microbial community in olive plants under different conditions

Italy  
Spain  
Portugal





# USE OF MICROBIAL ANTAGONIST

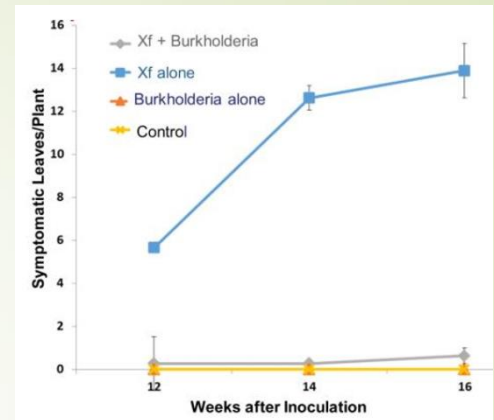
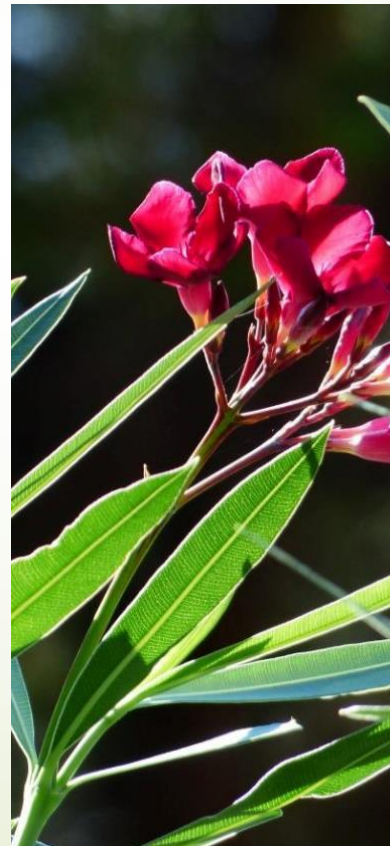
**PLANT GROWTH-  
PROMOTING  
RHIZOBACTERIUM**

**STIMULATION OF  
PLANT GROWTH**

**IMPROVEMENT  
OF TOLERANCE  
TO ABIOTIC  
STRESS**

**REDUCTION OF  
PLANT DISEASE  
SUSCEPTIBILITY**

**PROVED SUCCESSFUL FOR BIOLOGICAL  
CONTROL OF  
PIERCE'S DISEASE IN GRAPEVINE**



**Inoculations in olives  
and oleander**

**Host colonization and  
persistence**

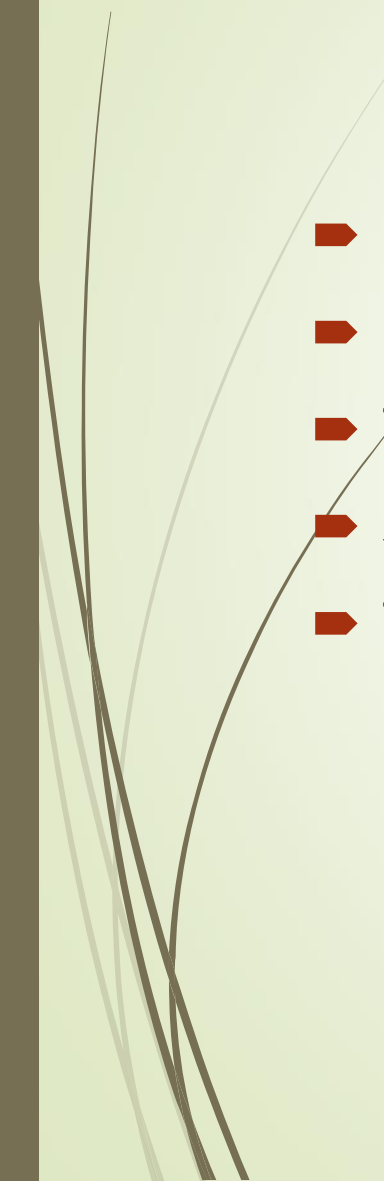
**Field experiments  
March 2018:**

- Curative treatment
- Prevention/pre-immunization





# CONTROL OF THE VECTORS

- COVER CROPS – JUVENILE REDUCTION
  - BORDER TRAP PLANTS
  - TESTING FORMULATES
  - SEARCHING FOR PARASSITOIDS AND PREDATORS
  - Trapping (acoustic signal)
- 

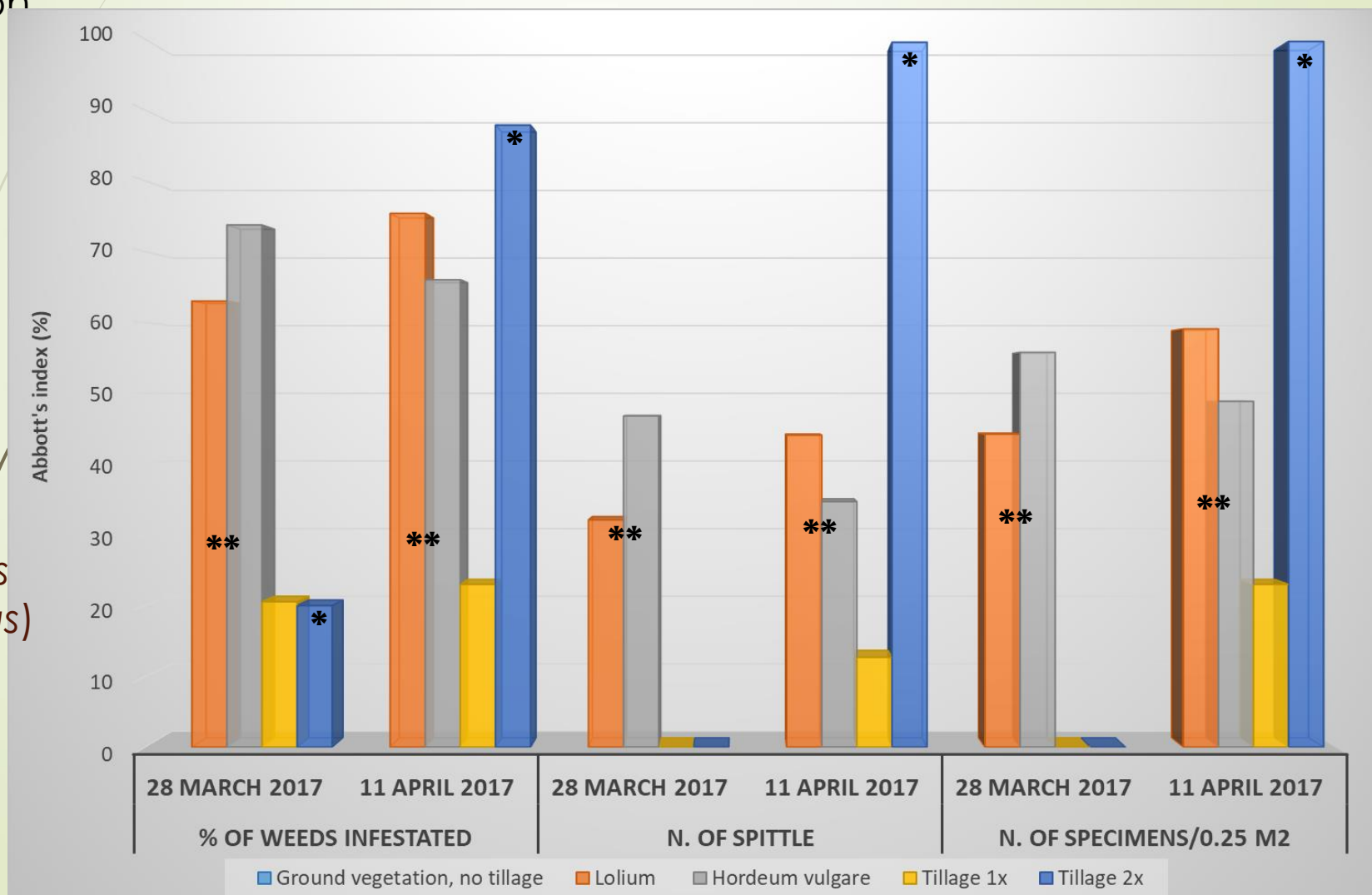


**\*Tilling the soil** twice (winter and spring ) provided the best results -  
reduction of spittle/specimens

**\*\*Sowing poaceae** (*Lolium* and *Hordeum*) contributed to reduce incidence of infestation

**TESTING  
COVER  
CROP to  
reduce  
juveniles**

(i.e. non-  
host of  
*Philaenus  
spumarius*)





## Task 7.3 – Evaluation of border plants as trap plants in olive orchards for the reduction of adults density population of *P. spumarius* (P1: CNR-IPSP/CRSFA)

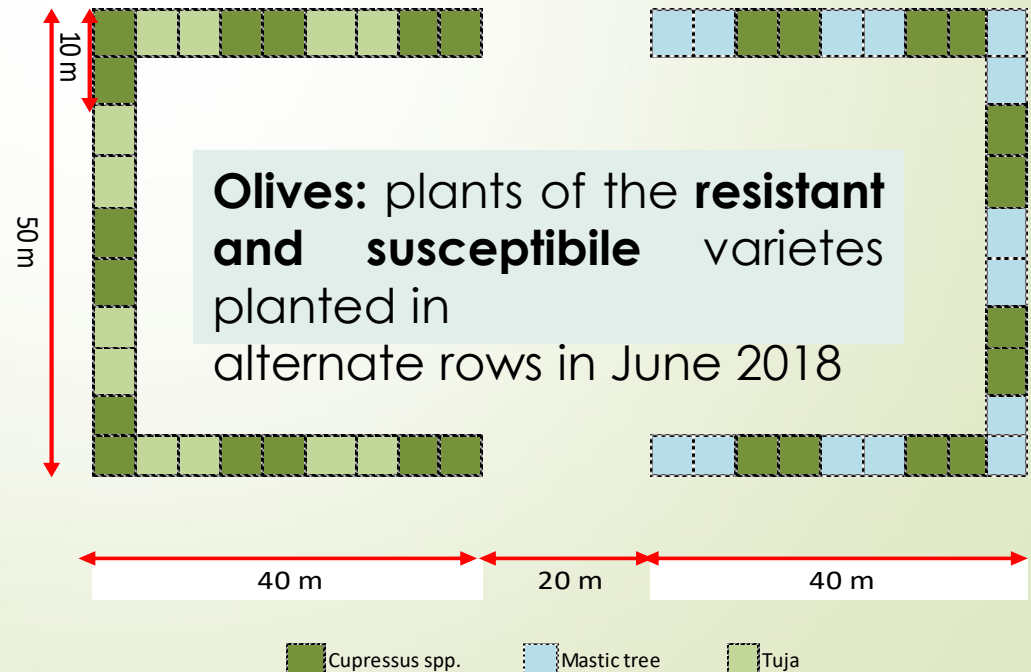
Border plants plated in 2017  
Olives: in 2018



Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy

Non-host *Xf pauca*, ST53

(**Thuja**, **Cupressus** spp. and **lentisk**) used in alternate rows and in different combinations, to set 3 field trials in the contaminated area.



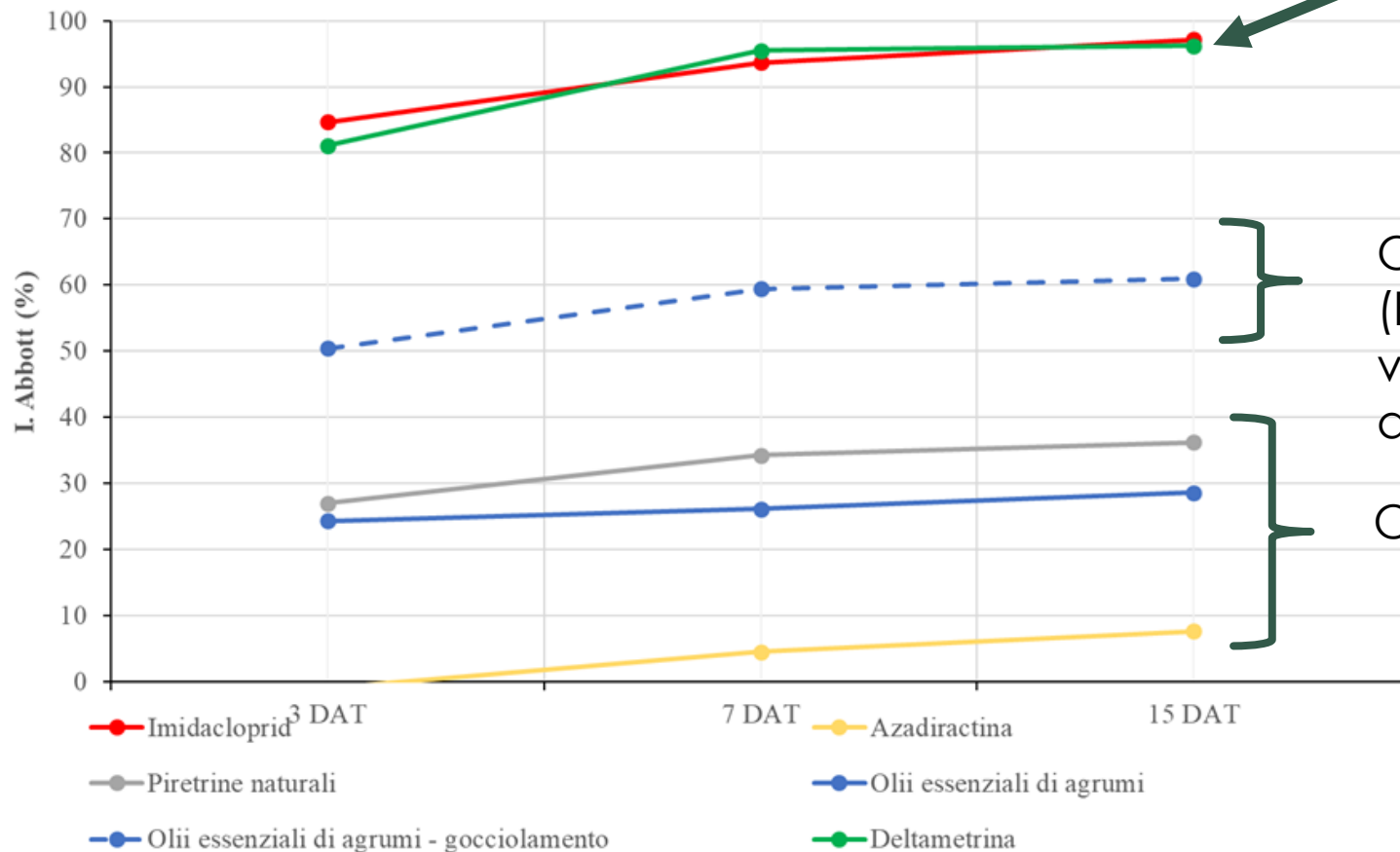


These data on efficacy of different chemicals and formulations to control *P. spumarius* showed that neonicotinoids performed better than the other products.



Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy

Example of the results in one of the trials



Neonicotinoids and Pyrethroids

Organic (PREVAM), high volume of application

Organic

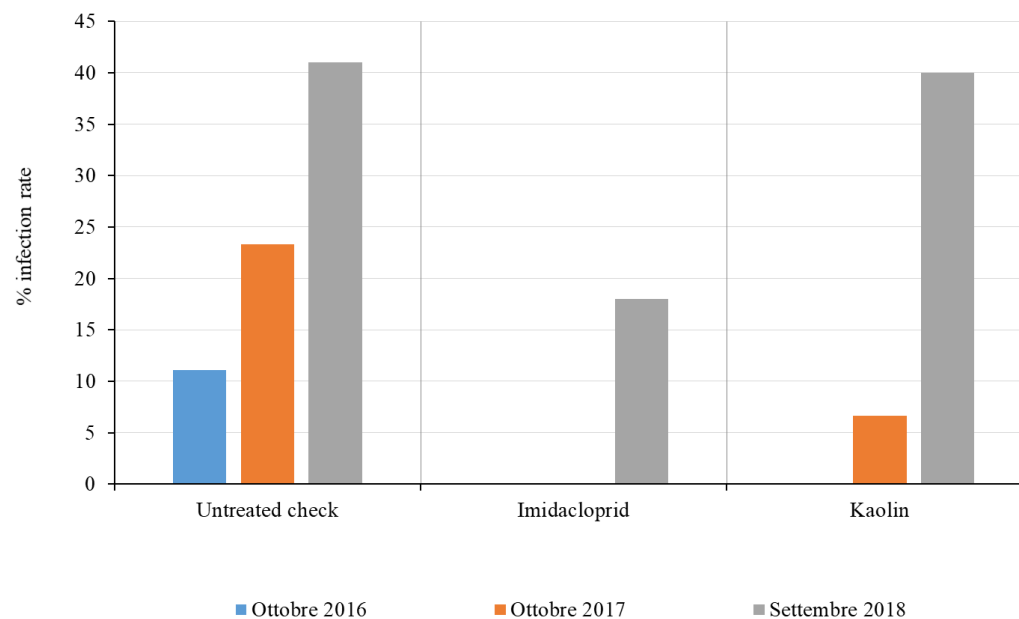




# Application of kaolin to reduce the spread of the infections in olives

New plantation  
in the  
contaminated  
area

Periodic surveys  
and sampling











# **PREVENTION**

- **Early detection**
  - **Producing Xf-free plants**
- 



# REMOTE SENSING SUPPORTING EARLY DETECTION

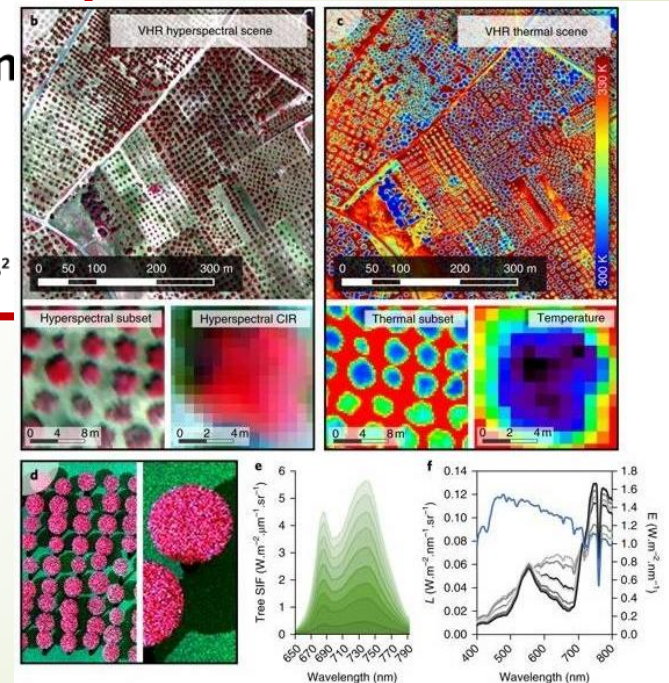
nature  
plants

LETTERS

<https://doi.org/10.1038/s41477-018-0189-7>

## Previsual symptoms of *Xylella fastidiosa* infection revealed in spectral plant-trait alterations

P. J. Zarco-Tejada<sup>1\*</sup>, C. Camino<sup>1b</sup>, P. S. A. Beck<sup>1</sup>, R. Calderon<sup>2</sup>, A. Hornero<sup>2,3</sup>,  
R. Hernández-Clemente<sup>3</sup>, T. Kattenborn<sup>4</sup>, M. Montes-Borrego<sup>2</sup>, L. Susca<sup>5</sup>, M. Morelli<sup>6</sup>,  
V. Gonzalez-Dugo<sup>2</sup>, P. R. J. North<sup>3</sup>, B. B. Landa<sup>1b,2</sup>, D. Boscia<sup>6</sup>, M. Saponari<sup>6</sup> and J. A. Navas-Cortes<sup>2</sup>







INITIATIVE within the project XF-ACTORS

## VSPP Voluntary System Preventing Pests

- Is **proposed in addition to** EU regulation
- Focus on *Xylella fastidiosa*
- Later to **be extended** to other pests

Naktuinbouw – CIVITALIA - EPPO



# Nursery productions under protected conditions



## Technical specifications



# Implementation of the project

48

## Engagement of fruitful collaborations with local stakeholders





# Implementation of the project

49

- **Delivery of science-based information to stakeholders:** several technical meetings with field visits have been organized (NPPO representatives, growers associations/organization, private companies: Bayer, Agromillora, Todolivo)





# Dissemination – Capacity building

50

- Workshops and training (diagnostics of the bacterium and insect vectors)





# Dissemination - Exploitation of the results

51

- Working group for the revision of the DP
- Working experts for the update of the EFSA scientific opinion





# European Research on *Xylella fastidiosa*

## 2nd Joint Annual Meeting

[info@ponteproject.eu](mailto:info@ponteproject.eu)  
[info@xfactorsproject.eu](mailto:info@xfactorsproject.eu)

Valencia (Spain) | 23-25 October 2018

**ivia**  
Instituto Valenciano  
de Investigaciones Agrarias



### Surveillance and early detection of *Xylella fastidiosa*



### Genomics, epidemiology and vectors



### Developing disease control strategies



Contributions of the H2020 projects  
**POnTE** and **XF-ACTORS**



# National and International projects Research network > 30 Institutions

