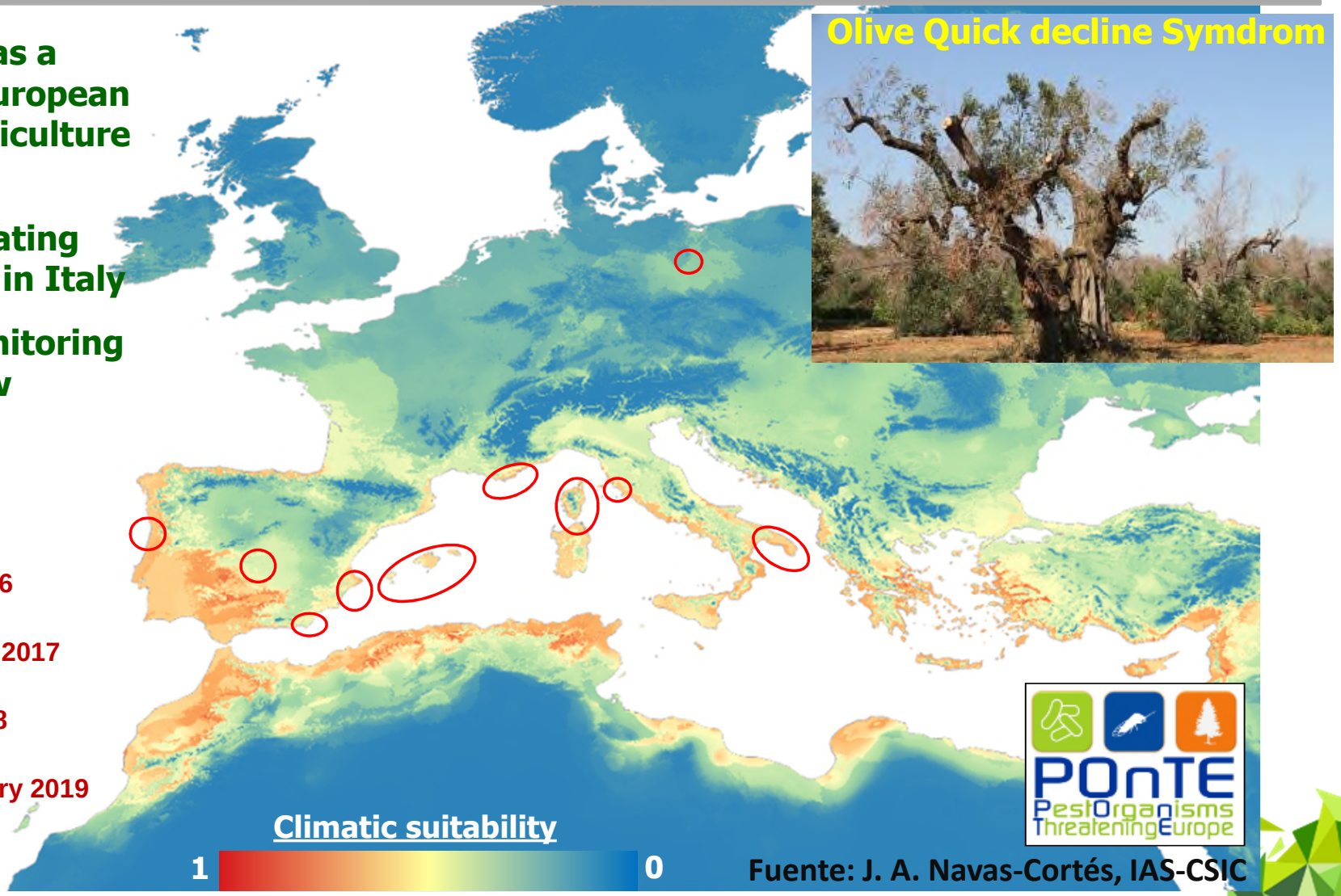
Understanding the potential origin and epidemiological consequences of the Spanish outbreaks caused by *Xylella fastidiosa* subsp. *multiplex*

Blanca B. Landa, Andreina Castillo Siri, Annalisa Giampetruzzi, Miguel Román, M. Pilar Velasco, Ester Marco-Noales, Eduardo Moralejo, Maria Saponari, Juan A. Navas-Cortés, Rodrigo Almeida

Outbreaks and detections of *Xylella fastidiosa* in Europe

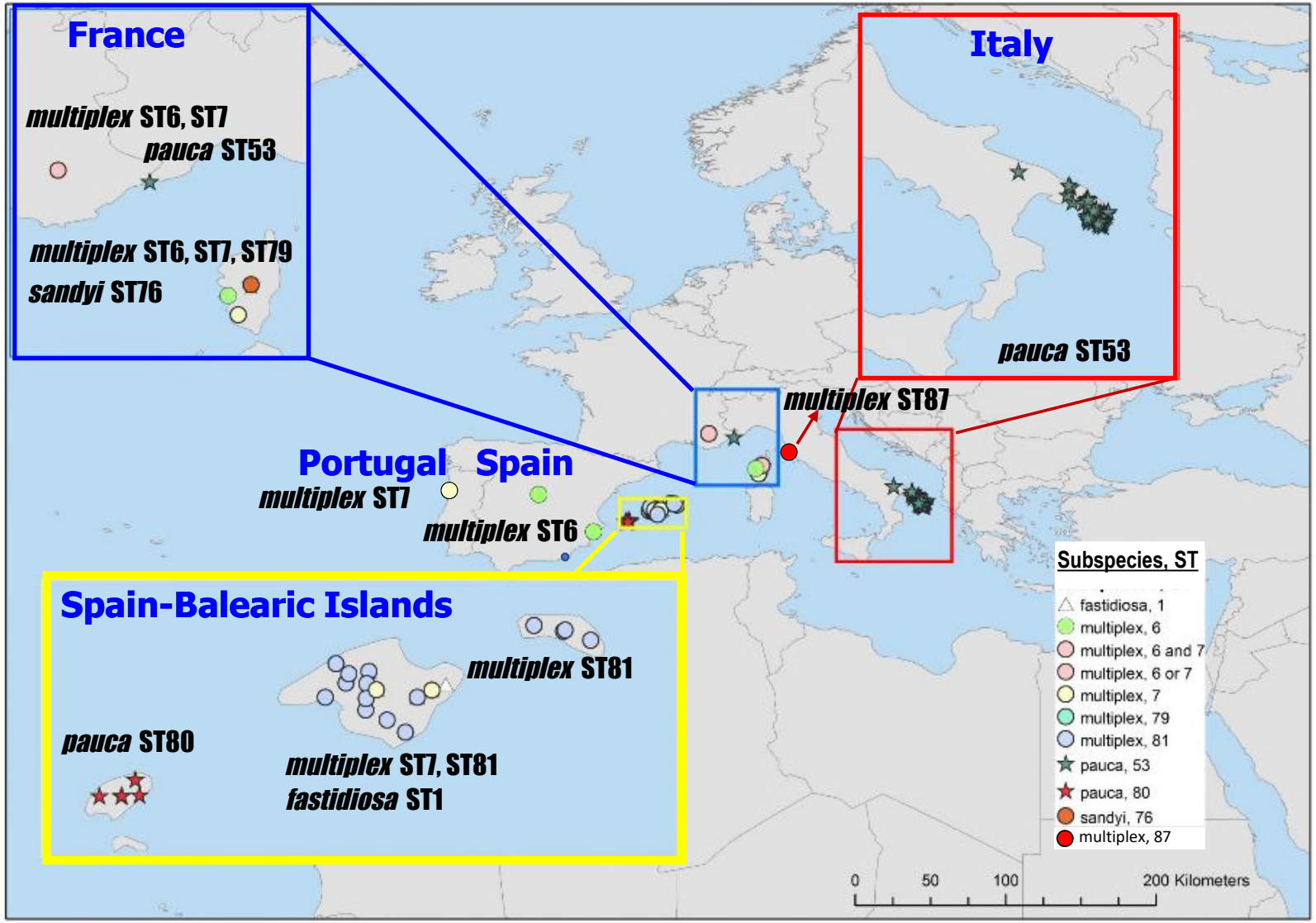
- ***X. fastidiosa*** **emerged as a serious threat to the European and Mediterranean agriculture and landscape in 2013**
- **Associated to a devastating epidemic in olive trees in Italy**
- **Official mandatory monitoring within EU revealed new outbreaks**

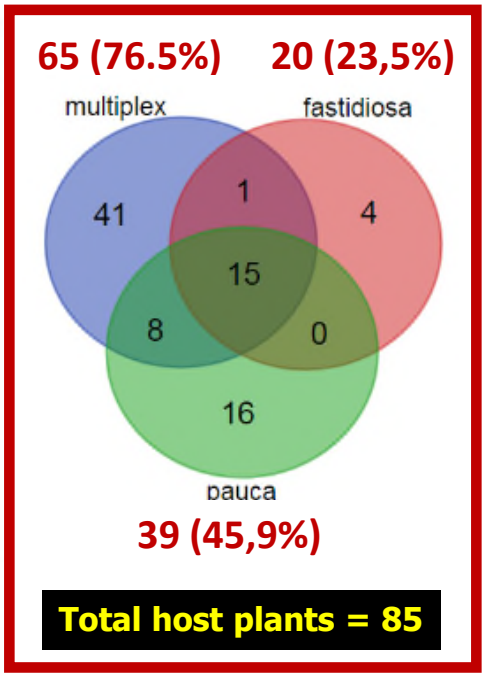
Apulia, Italy, October 2013
Corsica, France, July 2015
PACA, France, October 2015 & 2016
Saxony, Germany, April 2016
Mallorca, Spain, November 2016 & 2017
Alicante, Spain, July 2017
Madrid & Almería, Spain, April 2018
Tuscany, Italy, October 2018
Vila Nova de Gaia, Portugal, January 2019



Fuente: J. A. Navas-Cortés, IAS-CSIC

Genetic diversity of *Xylella fastidiosa* in Europe

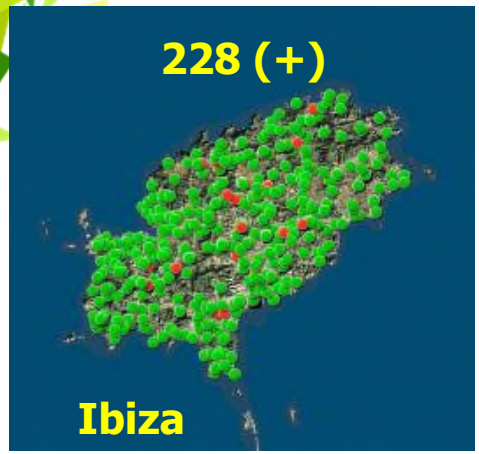




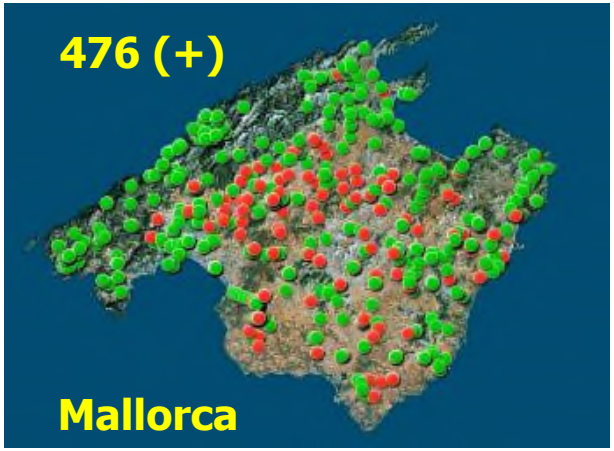
- ***Xf multiplex*** **accounts for the larger number of susceptible plant species (more than 75%)**

Adapted from EFSA Journal 2018; 16(7):5357, 61 pp.

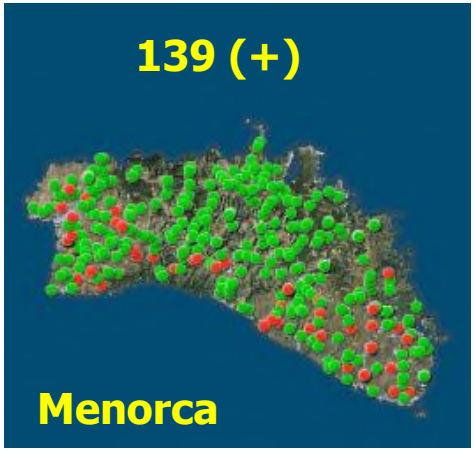
Situation of *Xylella fastidiosa* in Spain



Subsp. *pauca* ST80



Subsp. *fastidiosa* ST1
Subsp. *multiplex* ST7, ST81



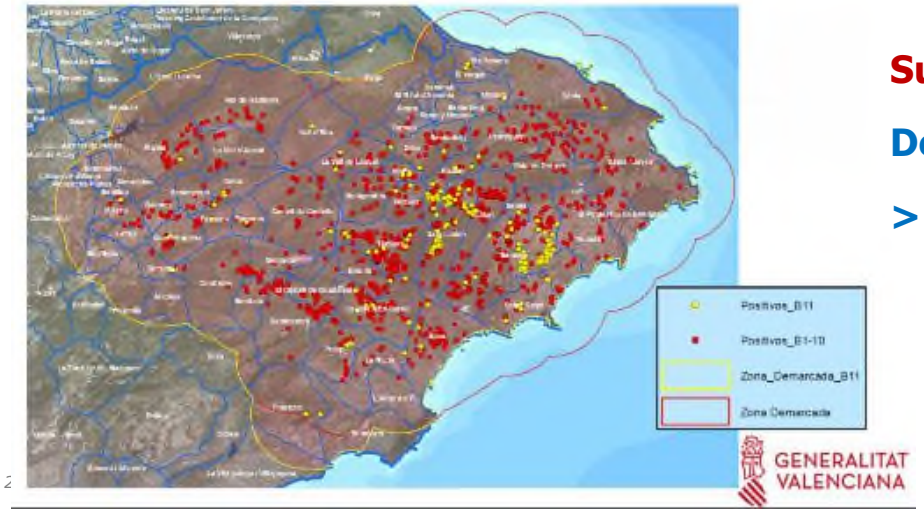
Subsp. *multiplex* ST81

Balearic Islands

1st notification
October 2016

Under containment
14 December 2017

843 positives (Feb. 2019)



Subsp. *multiplex* ST6

Demarcated área: > 138.000 ha

> 1500 positives in 74 Municipalities

Alicante

1st notification
June 2017

Under eradication

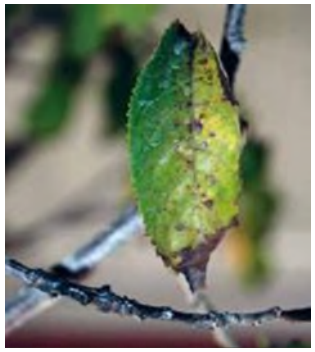
Situation of *Xylella fastidiosa* in the Balearic Islands

➢ **Host plants: 21 species of crops, ornamentals and natural vegetation**

Grapes
***fastidiosa* ST1**

Almond
***fastidiosa* ST1 / *multiplex* ST7,ST81**

Cherry
***fastidiosa* ST1**



Plum
***multiplex* ST81**

Polygala myrtifolia
All ST 1, ST81, ST7, ST80

Olive
***multiplex* ST81/ *pauca* ST80**

Oleaster
***multiplex* ST81/ *pauca* ST80**

Fig
***multiplex* ST81**

Walnut
Subsp. *fastidiosa* ST1

Nerium oleander
Subsp. ?

Situation of *Xylella fastidiosa* in the Balearic Islands

➢ **Host plants: 21 species of crops, ornamentals and natural vegetation**

Lavandula dentata
***multiplex* ST81 / *pauca* ST80**

Rosmarius officinalis
***multiplex* ST81**

Calicotome spinosa
***fastidiosa* ST1**

Cistus monspeliensis
***fastidiosa* ST1**

Cistus albidus
***multiplex* ST81 / *pauca* ST80**

Fraxinus angustifolia
***multiplex* ST81**

***Acacia* sp.**
***multiplex* ST81 / *pauca* ST80**

Rhamnus alaternus
***fastidiosa* ST1**

Genista lucida
***fastidiosa* ST1**

Teucrium capitatum
***fastidiosa* ST1**

Lavandula angustifolia
***multiplex* ST81**

Situation of *Xylella fastidiosa* in Alicante

➢ **Host plants: 12 species of crops, ornamentals and natural vegetation**

*Prunus dulcis***

*Prunus armeniaca***

*Prunus domestica***

X. fastidiosa* subsp. *multiplex* * ST6*

- **Positive samples > 1500**
- **Almond > 94% of positives**

*Helichrysum italicum**

*Polygala myrtifolia***

*Rosmarinus officinalis***

Lavandula dentata

Lavandula latifolia

Calicotome spinosa

*Phagnalon saxatile***

Rhamnus alaternus

Helichrysum stoechas

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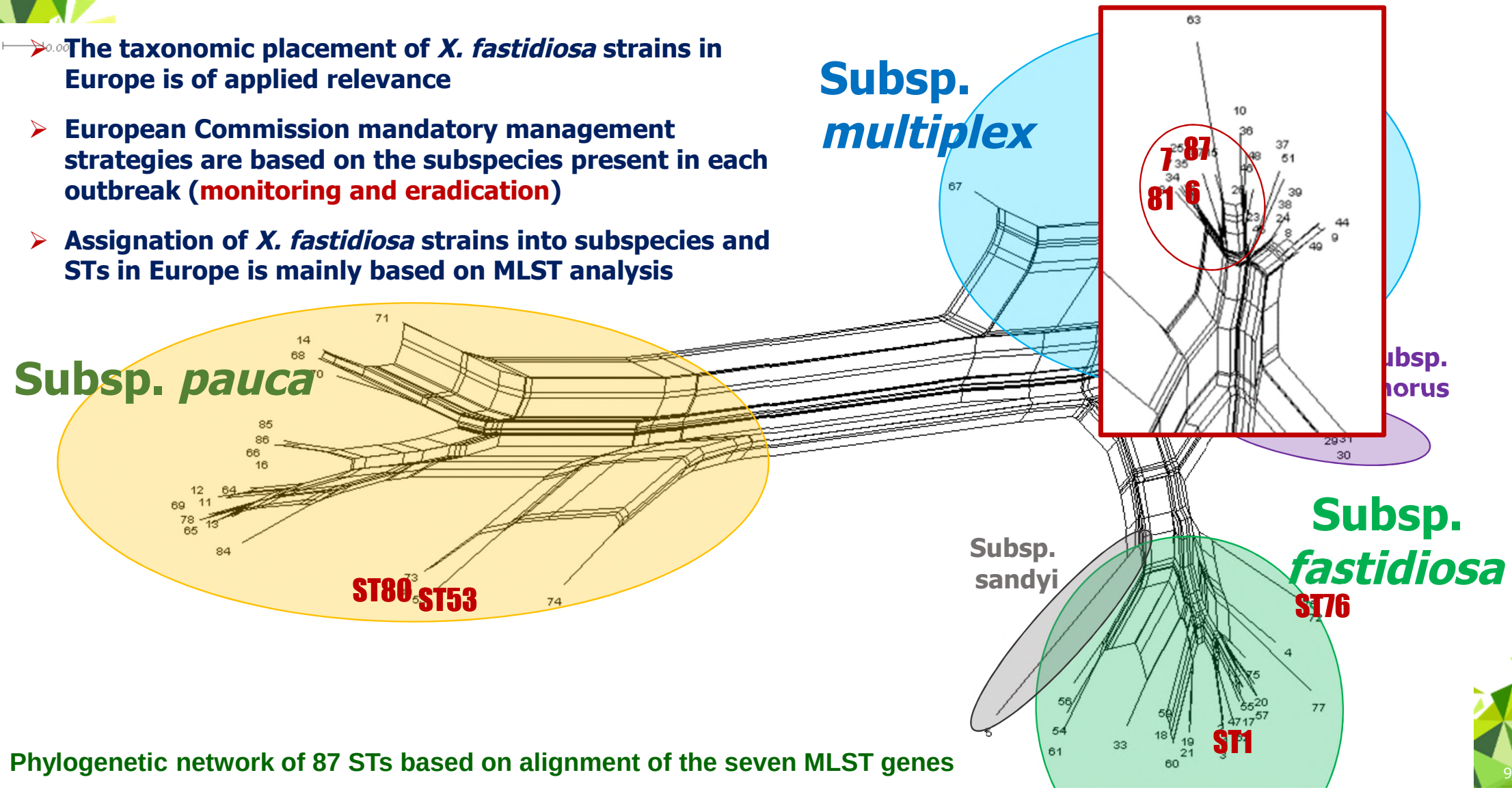
Host plants of *Xylella fastidiosa* subsp. *multiplex* in Spain

Region	N	Host plants
Alicante + Mallorca & Menorca	5	*Polygala myrtifolia* *Lavandula dentata* *Prunus domestica* *Prunus dulcis* *Rosmarinus officinalis*
Alicante	7	*Calicotome spinosa* *Helichrysum stoechas* *Prunus armeniaca* *Phagnalon saxatile* *Lavandula latifolia* *Rhamnus alaternus* *Helichrysum italicum*
Mallora & Menorca	7	*Acacia sp.* *Cistus albidus* *Ficus carica* *Fraxinus angustifolia* *Lavandula angustifolia* *Olea europaea* var. *europaea* *Olea europaea* var. *sylvestris*

No olive trees infected in Alicante

Genetic diversity of *Xylella fastidiosa* in Europe

- The taxonomic placement of *X. fastidiosa* strains in Europe is of applied relevance
- European Commission mandatory management strategies are based on the subspecies present in each outbreak (monitoring and eradication)
- Assignation of *X. fastidiosa* strains into subspecies and STs in Europe is mainly based on MLST analysis



Phylogenetic network of 87 STs based on alignment of the seven MLST genes

Genetic diversity of *Xylella fastidiosa* in Europe

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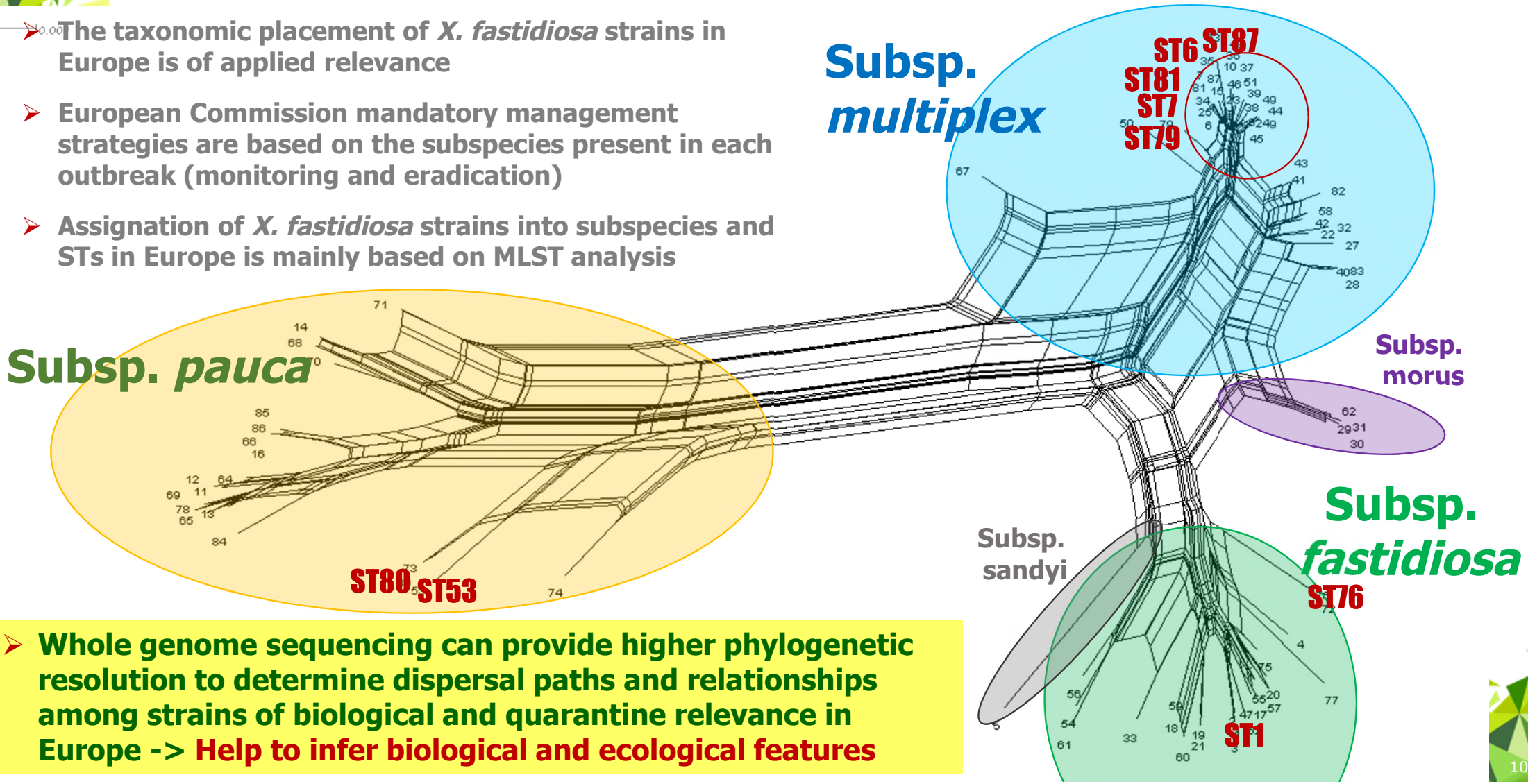


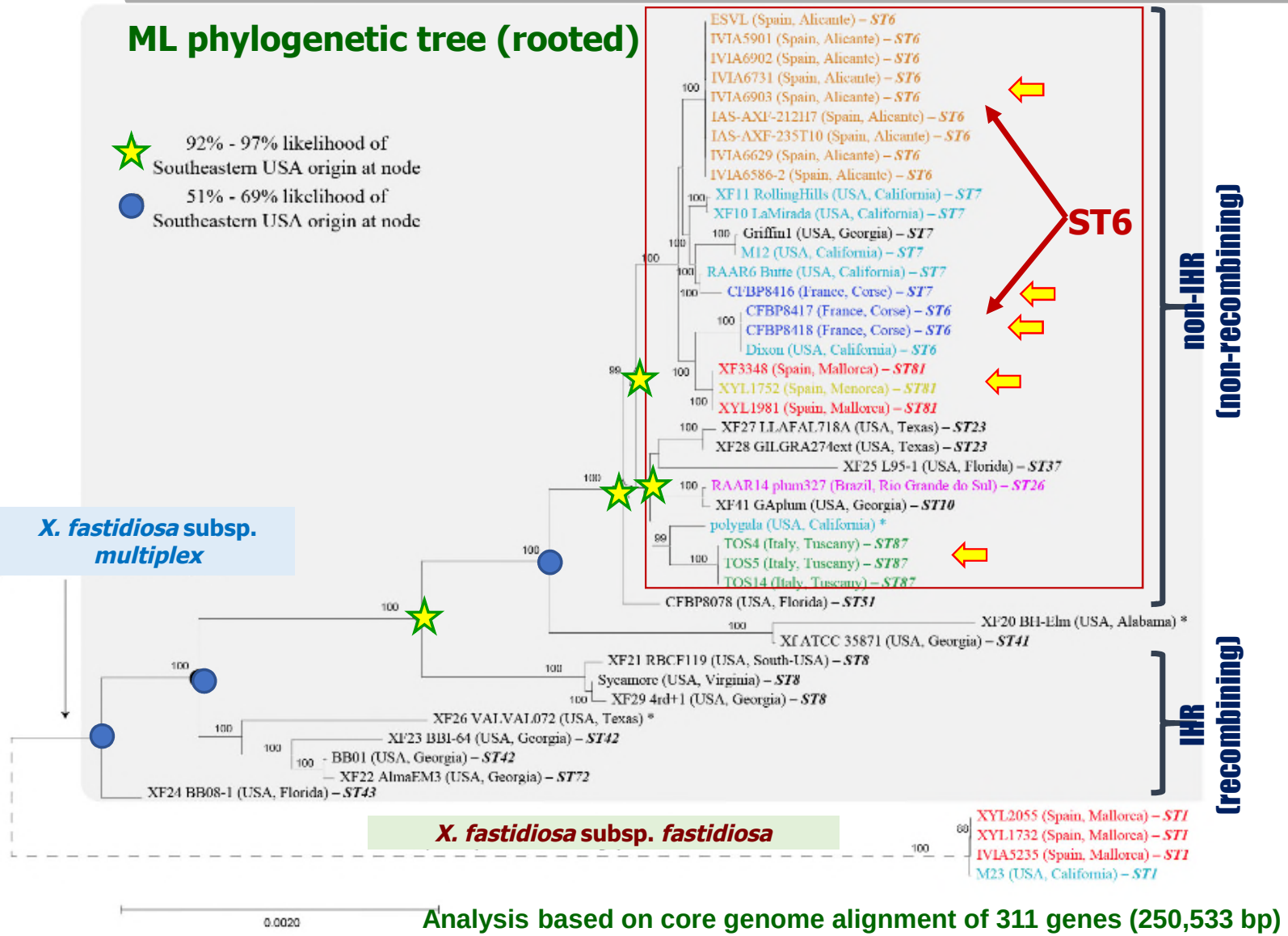
- **Whole genome sequencing can provide higher phylogenetic resolution to determine dispersal paths and relationships among strains of biological and quarantine relevance in Europe -> Help to infer biological and ecological features**

Genomes of european *Xylella fastidiosa* strains in the study

Isolate name	Subspecies	ST	Host	Geographical Origin	Year	Publication
XYL1732, XYL2055	*fastidiosa*	1	*Vitis vinifera*	Spain, Mallorca	2017	Gomila et al., 2019
IVIA5235	*fastidiosa*	1	*Prunus avium*	Spain, Mallorca	2017	Landa et al., 2018
CFBP8417, CFBP8418	*multiplex*	6	*Spartium junceum*	France, Corsica, Corse-du-Sud	2015	Denancé et al., 2017
ESVL, IVIA5901	*multiplex*	6	*Prunus dulcis*	Spain, Alicante	2017	Giampetruzzi et al., 2018
IVIA6586-2, IVIA6731	*multiplex*	6	*Helicrysum italicum*	Spain, Alicante	2018	This study
IAS-AXF-212H7, IVIA6902, IAS-AXF-235T10, IVIA6903	*multiplex*	6	*Prunus dulcis*	Spain, Alicante	2018	This study
IVIA6629	*multiplex*	6	*Rhamnus alaternus*	Spain, Alicante	2018	This study
CFBP8416	*multiplex*	7	*Polygala myrtifolia*	France, Corsica, Corse-du-Sud	2015	Denancé et al., 2017
XF3348	*multiplex*	81	*Prunus dulcis*	Spain, Mallorca	2018	This study
XYL1981	*multiplex*	81	*Ficus carica*	Spain, Mallorca	2018	This study
XYL1752	*multiplex*	81	*Prunus dulcis*	Spain, Menorca	2018	This study
TOS4	*multiplex*	87	*Prunus dulcis*	Italy, Tuscany	2018	Giampetruzzi et al., 2019a
TOS5	*multiplex*	87	*Polygala myrtifolia*	Italy, Tuscany	2018	Giampetruzzi et al., 2019a
TOS14	*multiplex*	87	*Spartium junceum*	Italy, Tuscany	2018	Giampetruzzi et al., 2019a

- **18 partial genomes from subspecies *multiplex* (10 new) belonging to four STs (6, 7, 81 and 87)**
- **15 strains from Spain (3 strains from subsp. *fastidiosa*), 3 from Italy and 3 from France**
- **From eight host plants (cultivated crops, ornamental and spontaneous vegetation)**
- **Compared to 37 available genomes from subspecies *multiplex* (Europe, USA and Brazil)**

Evolutionary relationships between European/American *X. fastidiosa* populations



Analysis based on core genome alignment of 311 genes (250,533 bp)

Geography-based ancestral state reconstruction analysis suggested:

- ***X. fastidiosa* subsp. *multiplex* originated from the Southeastern USA**
- **Isolates from California, Europe and Brazil are consequence of introductions**

Five introduction events in Europe:

- **Italy (ST87)**
- **Corsica (ST6 and ST7)**
- **Spain-Balearic Islands (ST81)**
- **Spain-Alicante (ST6)**

Lack of monophyly for isolates assigned to ST6 by MLST analysis:

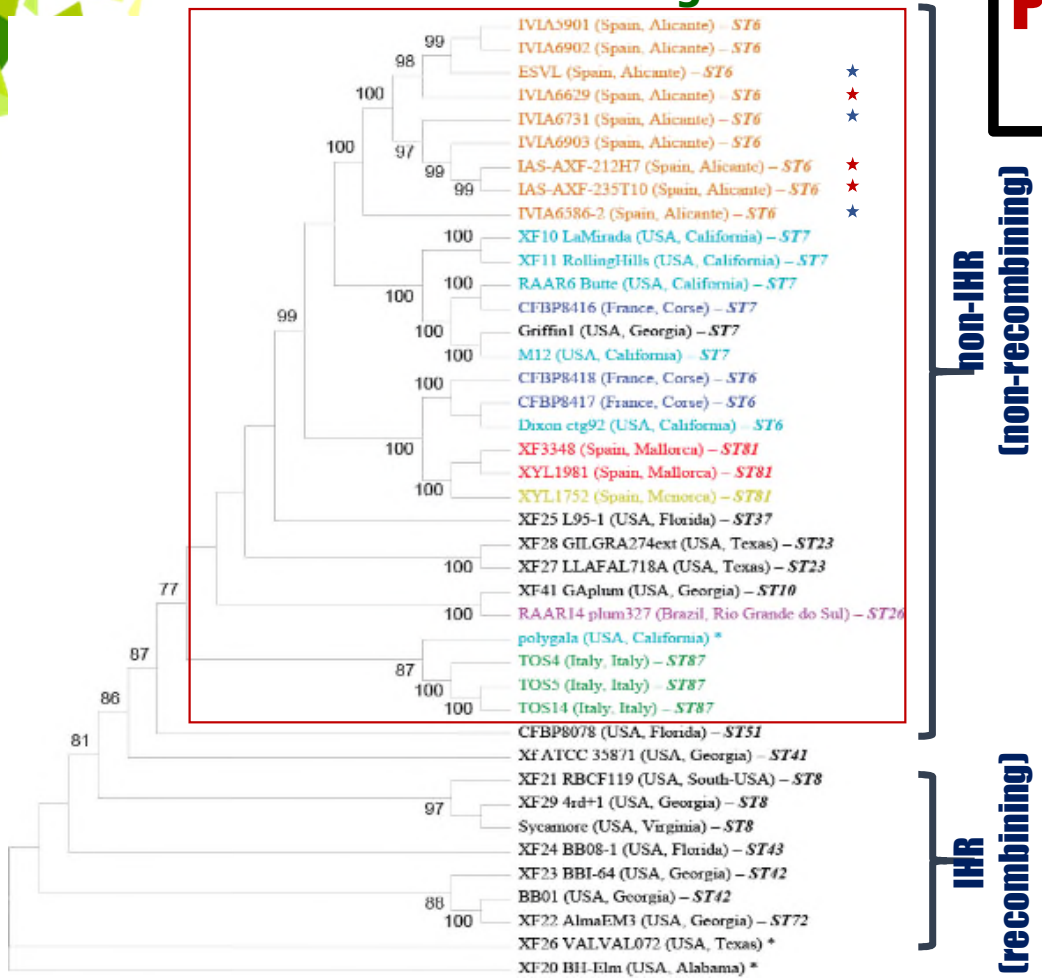
- **Alicante & Corsica**

European isolates belong to a relative young clade with limited intersubspecific recombination

- **The 'non-IHR group' (Nunney 2014)**

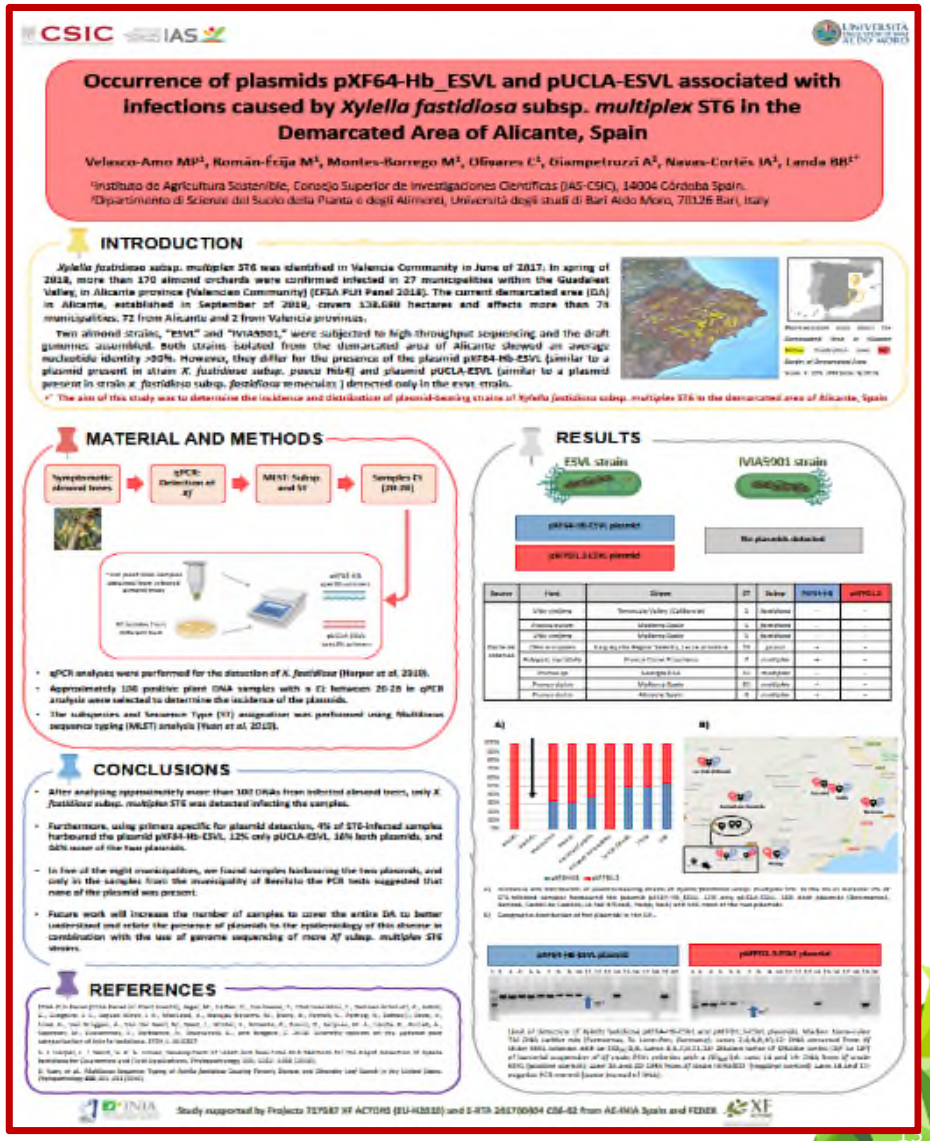
Evolutionary relationships between European/American *X. fastidiosa* populations

ML unrooted cladogram



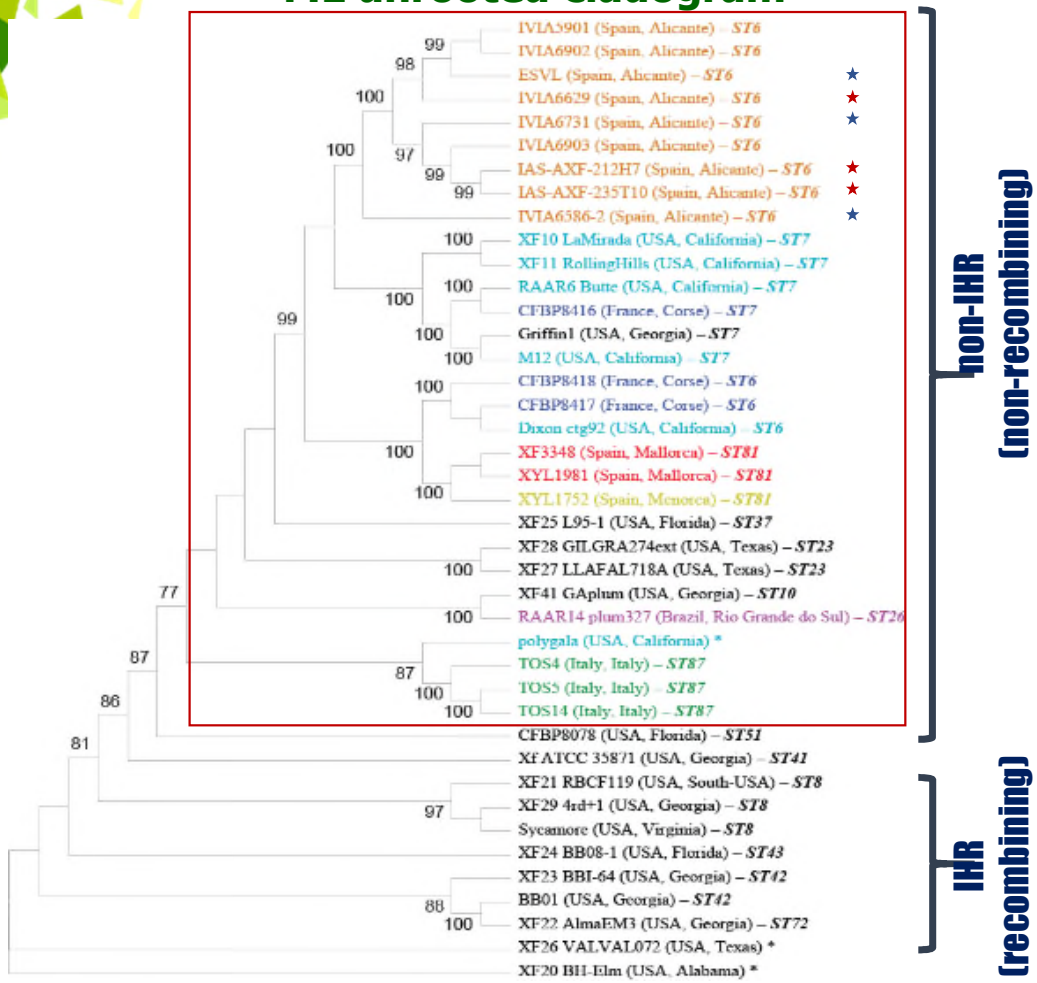
Based on core genome alignment of 348 genes (180,549 bp)
After removal of recombinant regions

POSTER P22



Evolutionary relationships between European/American *X. fastidiosa* populations

ML unrooted cladogram



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Session 8: Biology and Pathogenicity II

UNIVERSIDAD D CORDOBA

CSIC

INSTITUTO DE AGRICULTURA SOSTENIBLE IAS

AUBURN UNIVERSITY
COLLEGE OF AGRICULTURE

Phenotypic characterization of two Spanish strains of *Xylella fastidiosa* subsp. *multiplex* ST6 differing in plasmid content

cost
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

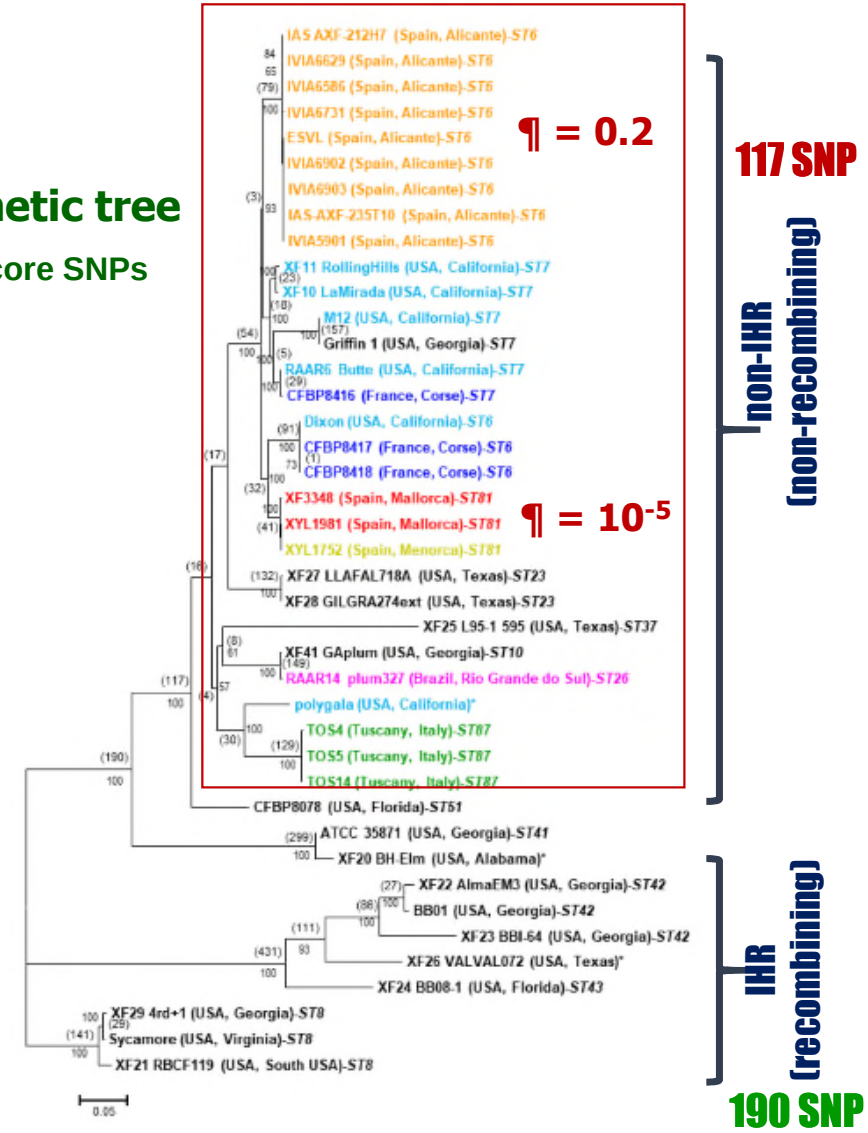
XF ACTORS

Miguel Román Écija
Institute for Sustainable Agriculture
Córdoba (Spain)

Evolutionary relationships between European/American *X.fastidiosa* populations

ML phylogenetic tree

Based on 5630 core SNPs



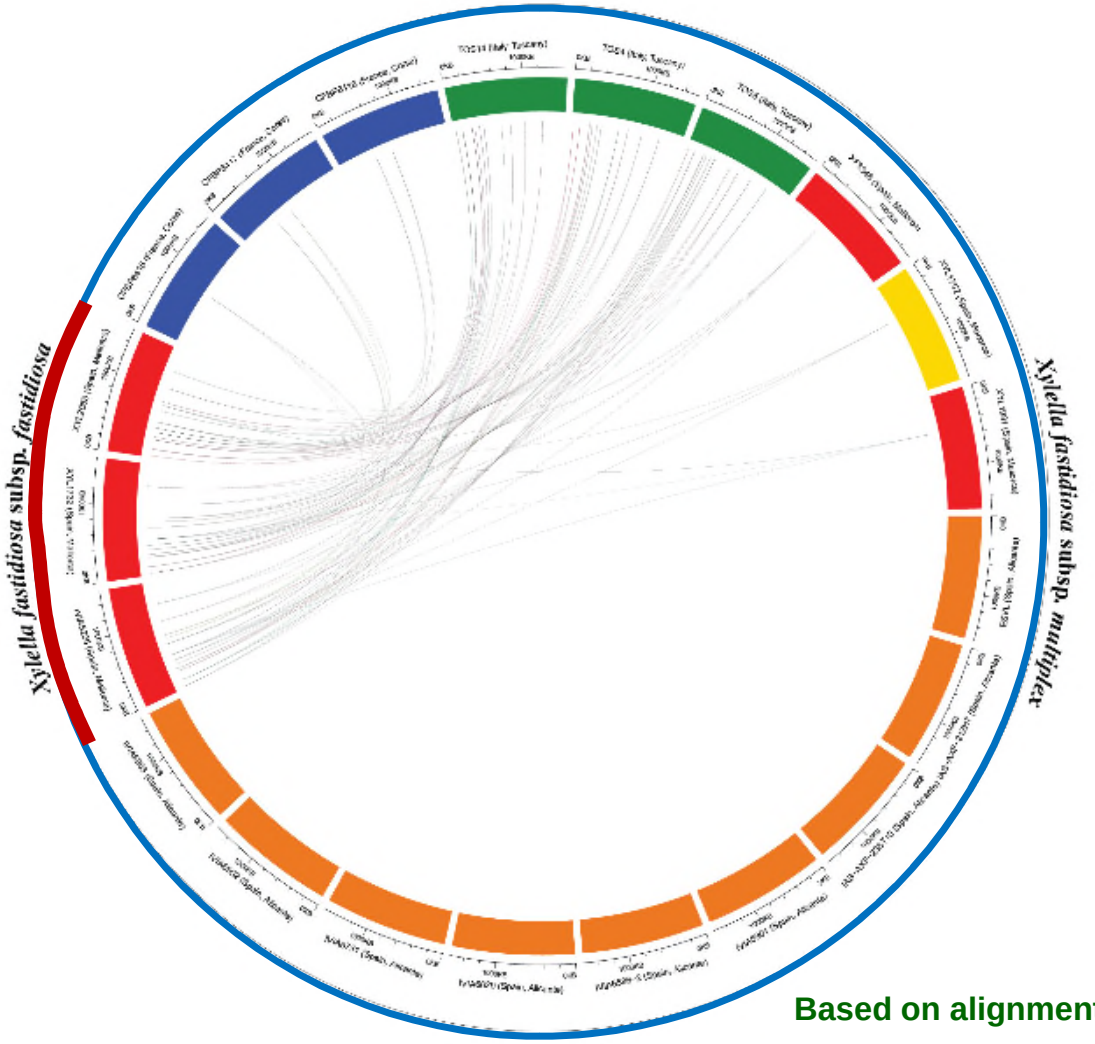
- **Number of SNPs and nucleotide diversity in European populations are indicative of:**
  - **Recent introductions**
  - **A relative young clade**

Origin	Shared SNPs within the cluster	Differentiating SNPs within the cluster
Alicante	79	10
Balearic Islands	41	3
Tuscany	129	1

Nucleotide diversity (¶)	California	Europe	Southeastern USA
California	59.53		
Europe	60.06	54.27	
Southeastern	289.21	293.81	354.40

Evolutionary relationships between European *X. fastidiosa* populations

Frequency of recombination events and their location along the length of the core genome alignment



- ***X. fastidiosa fastidiosa*** **from Mallorca shared the highest number of recombination events with *X. fastidiosa multiplex* strains from:**
  1. **Tuscany, Italy**
  2. **Balearic Islands, Spain**
  3. **Corsica, France**

  +

  -
- **There were more evidence of recombination events between allopatric than sympatric populations**
- **No recombinant events were detected with genomes from Alicante**
- **The recombination events were identified as 'recent'**
- **Most recombinant genes are hypothetical proteins of unknown function**

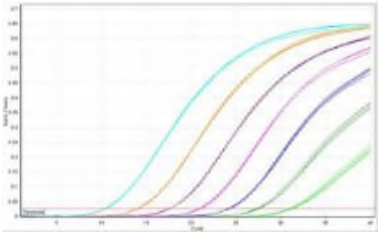
Based on alignment of 1588 genes, 1,569,508 bp

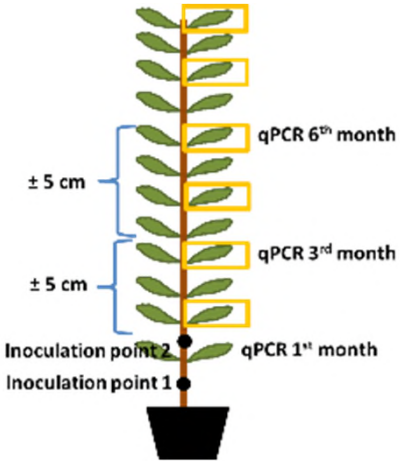
Pathogenicity experiments in olive (Spain)
X. fastidiosa pauca (ST53, ST80) and *X. fastidiosa multiplex* (ST6, ST81)

Olive Genotypes	**Picual, Hojiblanca y Arbequina**
Strains subsp. *multiplex* Strains subsp. *pauca*	**IVIA5901 & ESVL (ST6) & 1966 (ST81) De Donno (ST53) & 1961 (ST80)**
Experiments	**2 experiments: 180 plant inoculated**

Disease monitoring and Infection process:

- **Symptoms development** over time (weekly)
- **Bacterial infection process** (Duplex qPCR Harper + Ouyang)
- **Plant phenotyping (Physiological parameters):** Photosynthetic rate, Dry stress, pigment content, spectral signature, Nitrogen Balance Index, etc.





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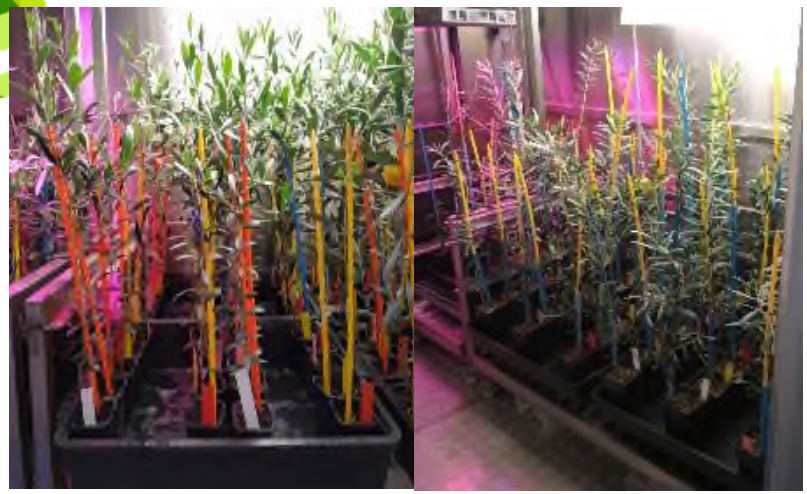
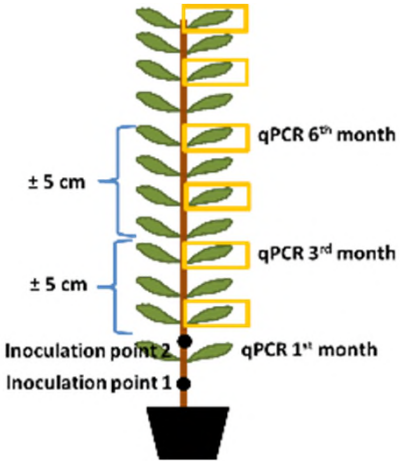
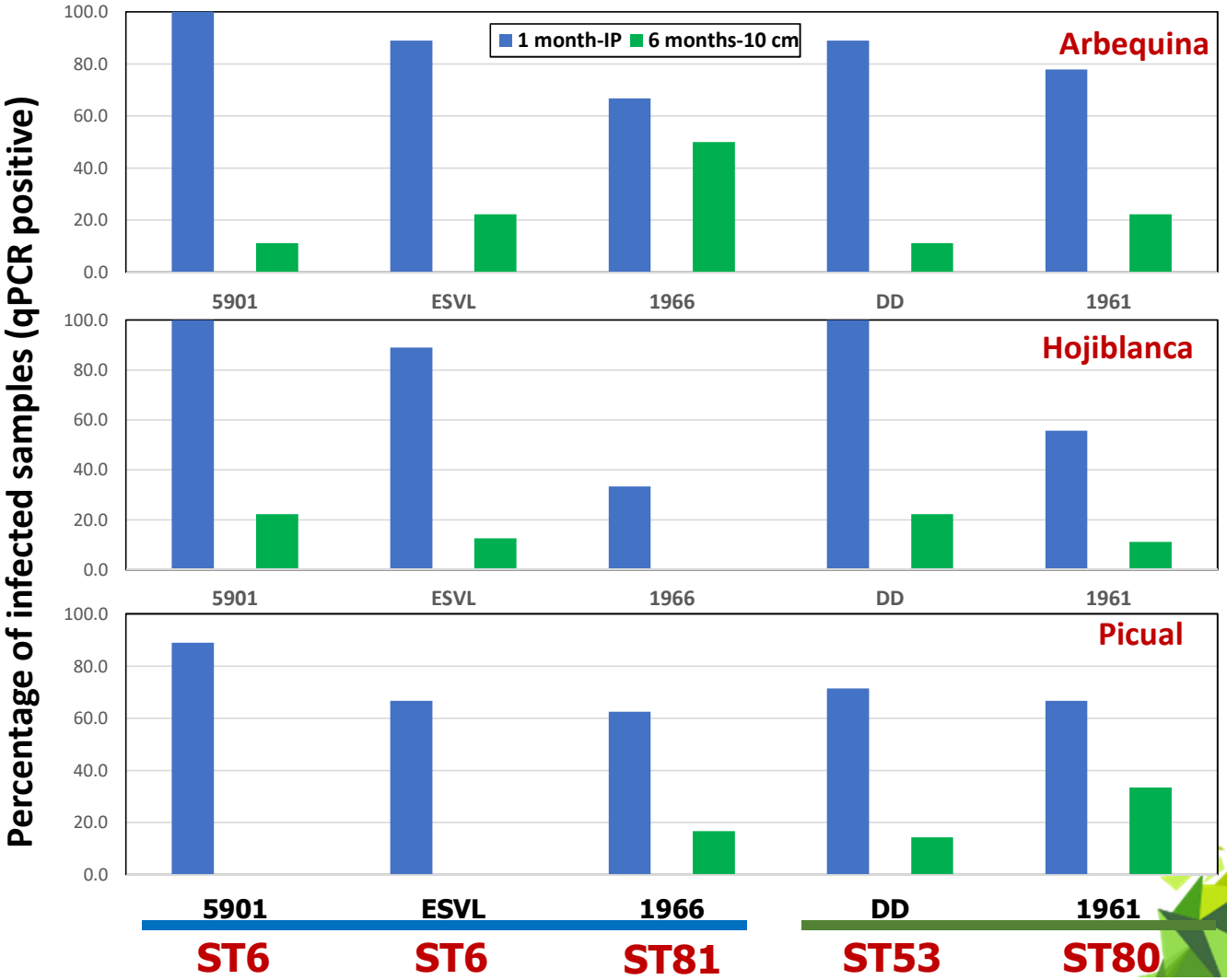




No symptoms developed after seven months



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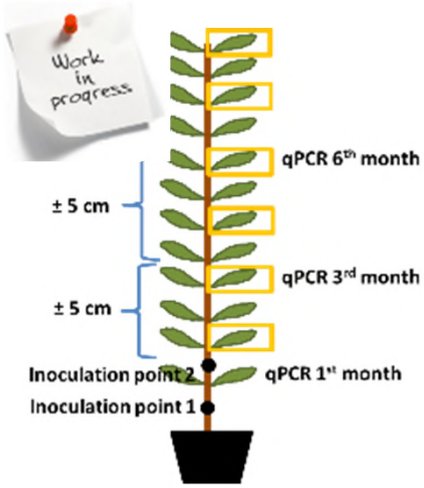
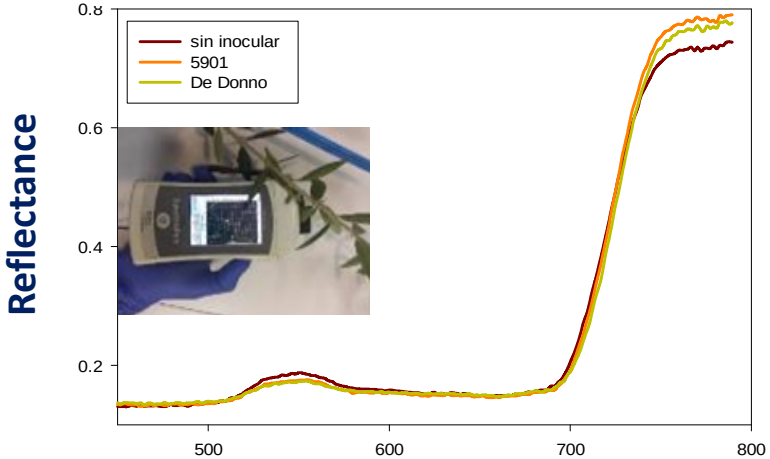
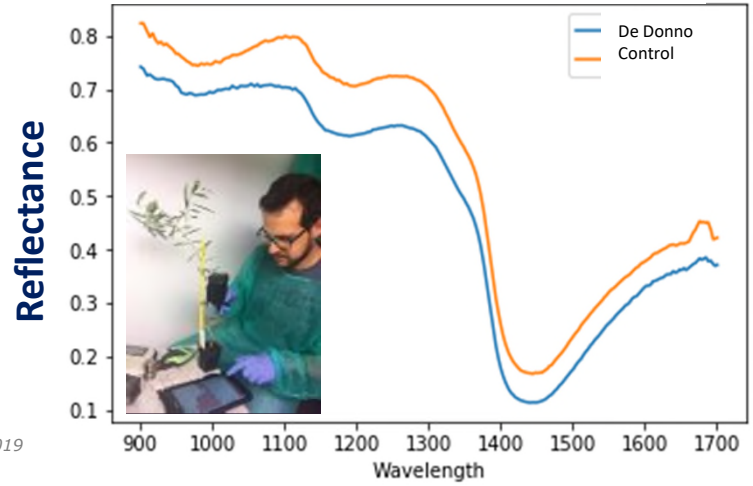
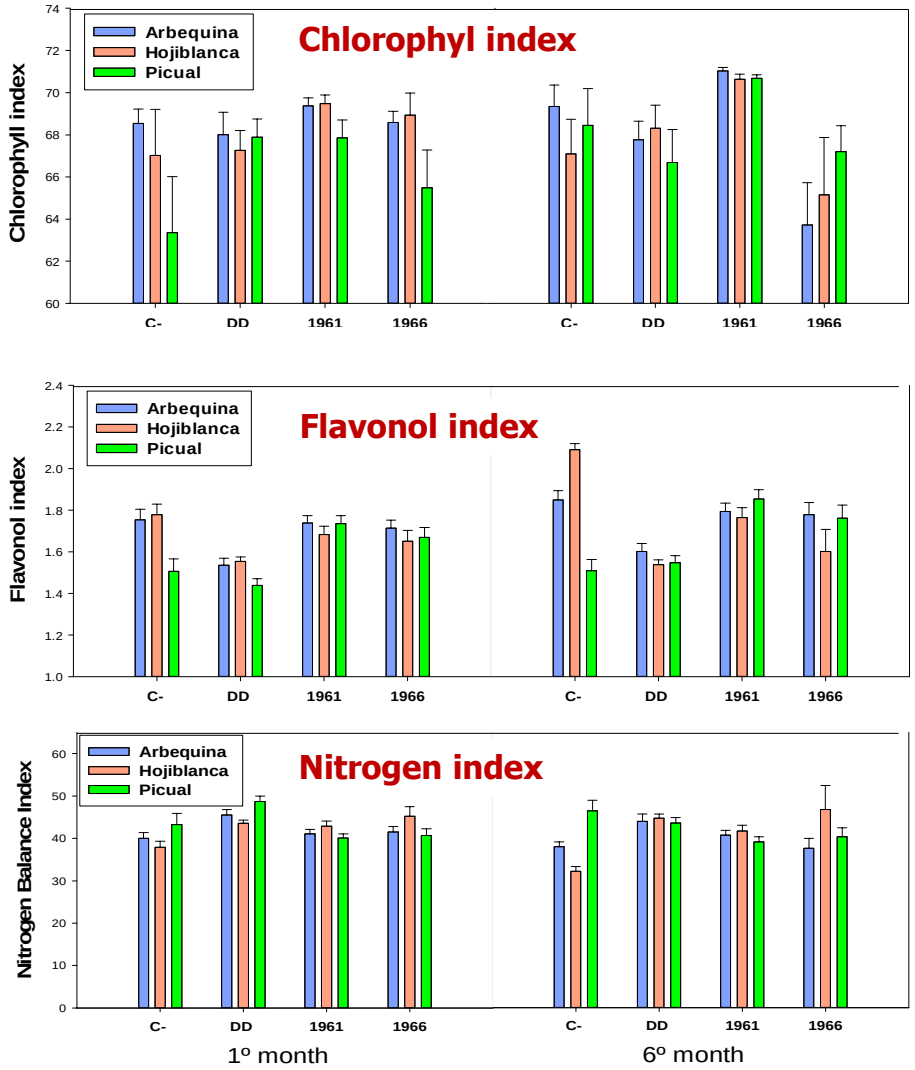


Spectral signature (450-800 nm)



Spectral signature (900-1700 nm)





2nd European Conference on Xylella fastidiosa 2019

Conclusions

- STs described by MLST analysis are not monophyletic
- *X. fastidiosa* subsp. *multiplex* has been introduced multiple times into Europe. It appears that most introductions originated from California
- There is evidence of recombination between *X. fastidiosa* sympatric populations, but there is no conclusive evidence that this recombination occurred in Europe
- There is a need to use whole-genome sequences to study pathogen introductions at outbreak stages to provide sufficient phylogenetic resolution. This can help to determine paths of dispersal or relationships among strains that are of biological and quarantine relevance

Overall, our work illustrates the risks associated with the commercial trade of plant material at global scales, and the need to develop effective policy to limit the likelihood of pathogen pollution into naïve regions

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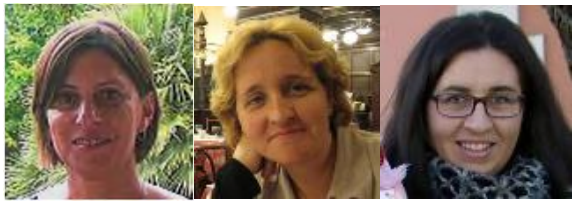

Maria Saponari, Donato Boscia, Annalisa Gimpetruzzi, Vito Montilon, Pasquale Saldarelli,

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Leonardo De la Fuente, Helvetio Coletta-Filho