Emergence of *Xylella fastidiosa* in Spain: current situation

Blanca B. Landa
Research Scientist
Institute for Sustainable Agriculture-CSIC
Córdoba, Spain

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MINISTERIO DE ECONOMÍA, INDUSTRIA Y COMPETITIVIDAD
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Process for oficial diagnosis of *Xylella fastidiosa* in Spain

GOVERN ILLES BALEARS

Official Plant Health Laboratory from Balearic islands

(+)

Autonomous community region level

Official Plant Health Laboratory from Comunitat Valenciana

(+)

GENERALITAT VALENCIANA
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GOBIERNO DE ESPAÑA MINISTERIO DE AGRICULTURA Y PESCA, ALIMENTACIÓN Y MEDIO AMBIENTE

ivia Instituto Valenciano de Investigaciones Agrarias

National Reference Laboratory for Phytopathogenic Bacteria

National level

- **Confirmation of Diagnosis**
- **Training and support**

(+)

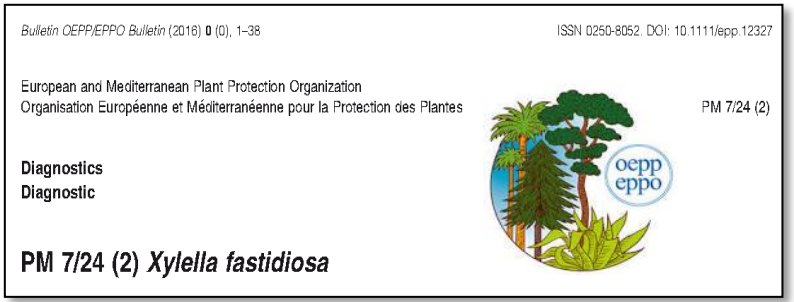
CSIC Consejo Superior de Investigaciones Científicas | Instituto de Agricultura Sostenible IAS

Laboratory of Biology & Ecology of Soil Microbiota IAS-CSIC

National level

- **Subspecies characterization**

- **Official surveys and monitoring**
- **Contingency Plan Implementation**


Bulletin OEPP/EPPO Bulletin (2016) **0** (0), 1–38 ISSN 0250-8052. DOI: 10.1111/epp.12327

European and Mediterranean Plant Protection Organization
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PM 7/24 (2)

Diagnostics
Diagnostic

PM 7/24 (2) *Xylella fastidiosa*

oepp eppo

Situation of *Xylella fastidiosa* in the Balearic islands

➢ **First outbreak: October 2016 in a garden center near Manacor city**

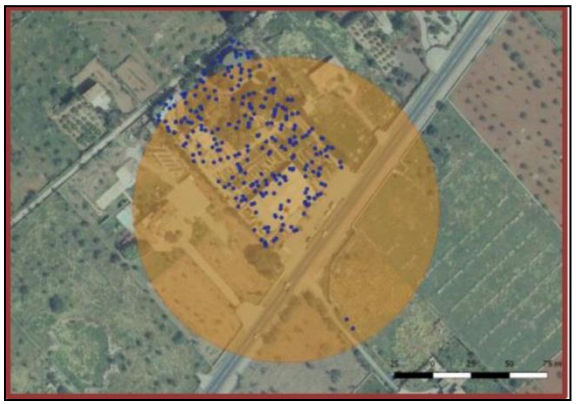
Official surveys Decision EU 2015/789

Atypical symptoms

3 Cherry samples (+)

Typical symptoms

Infected zone



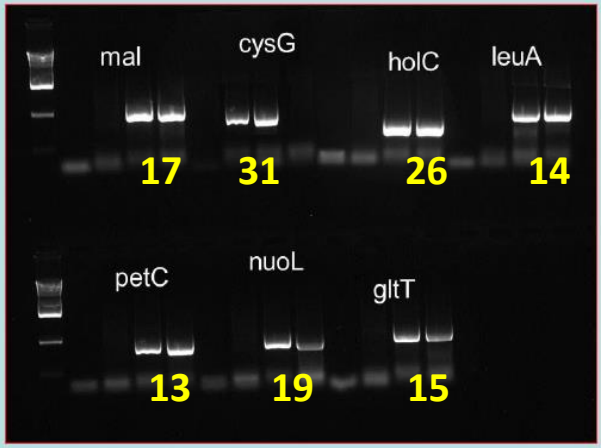
- **258 samples analyzed**
- **1921 host plants eradicated**

4 samples *Polygala myrtifolia* (+)

Situation of *Xylella fastidiosa* in the Balearic islands

➢ Identification of *X. fastidiosa* subspecies and STs in 1st outbreak

MLST analysis



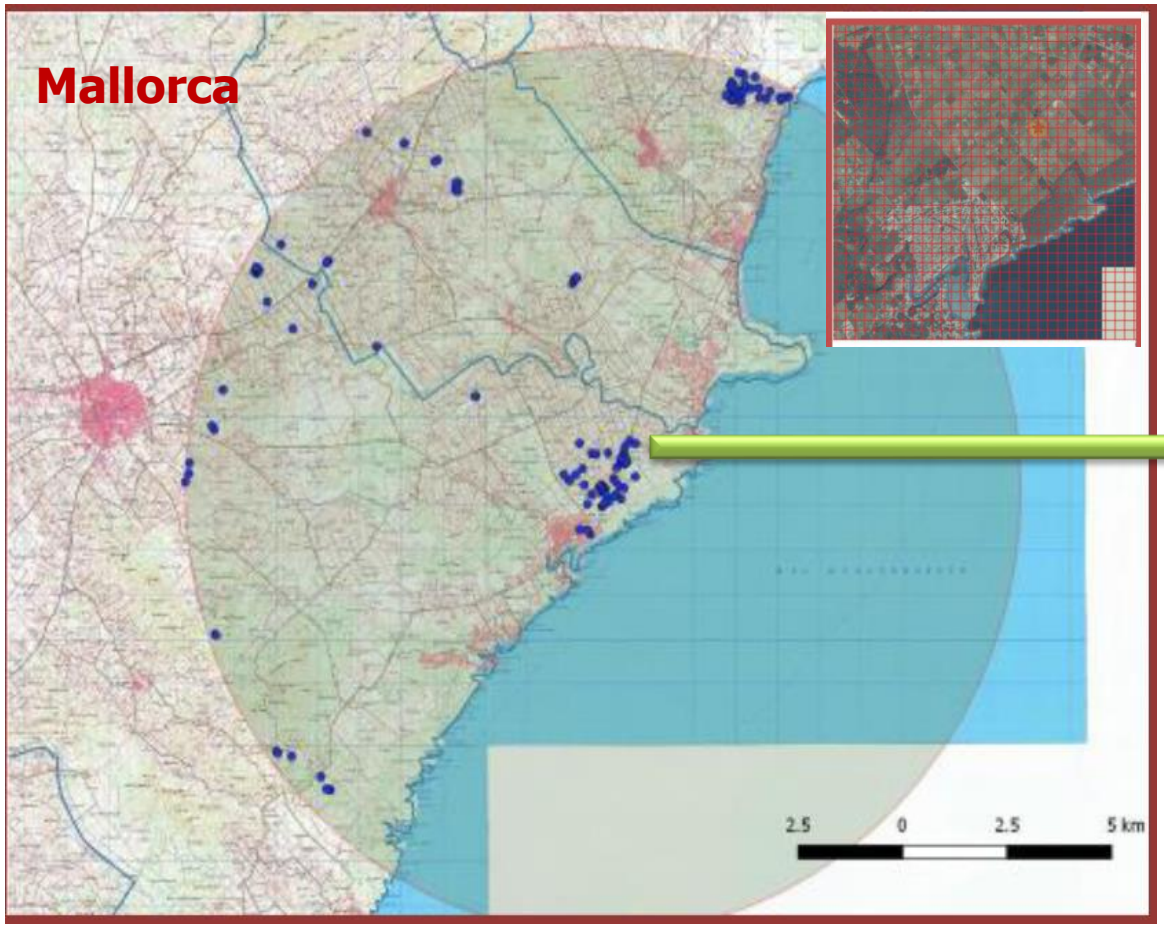
pilU and rpoD genes

ST	leuA	petC	malF	cysG	holC	nuoL	gltT
1	1	1	1	1	1	1	1

Host	#plants	Subspecies/ST
Cherry	3	*Xf* subsp. *fastidiosa* ST1
Polygala myrtifolia	3	*Xf* subsp. *fastidiosa* ST1

Situation of *Xylella fastidiosa* in the Balearic islands

➢ Contingency plan: Monitoring of the territory in the buffer zone



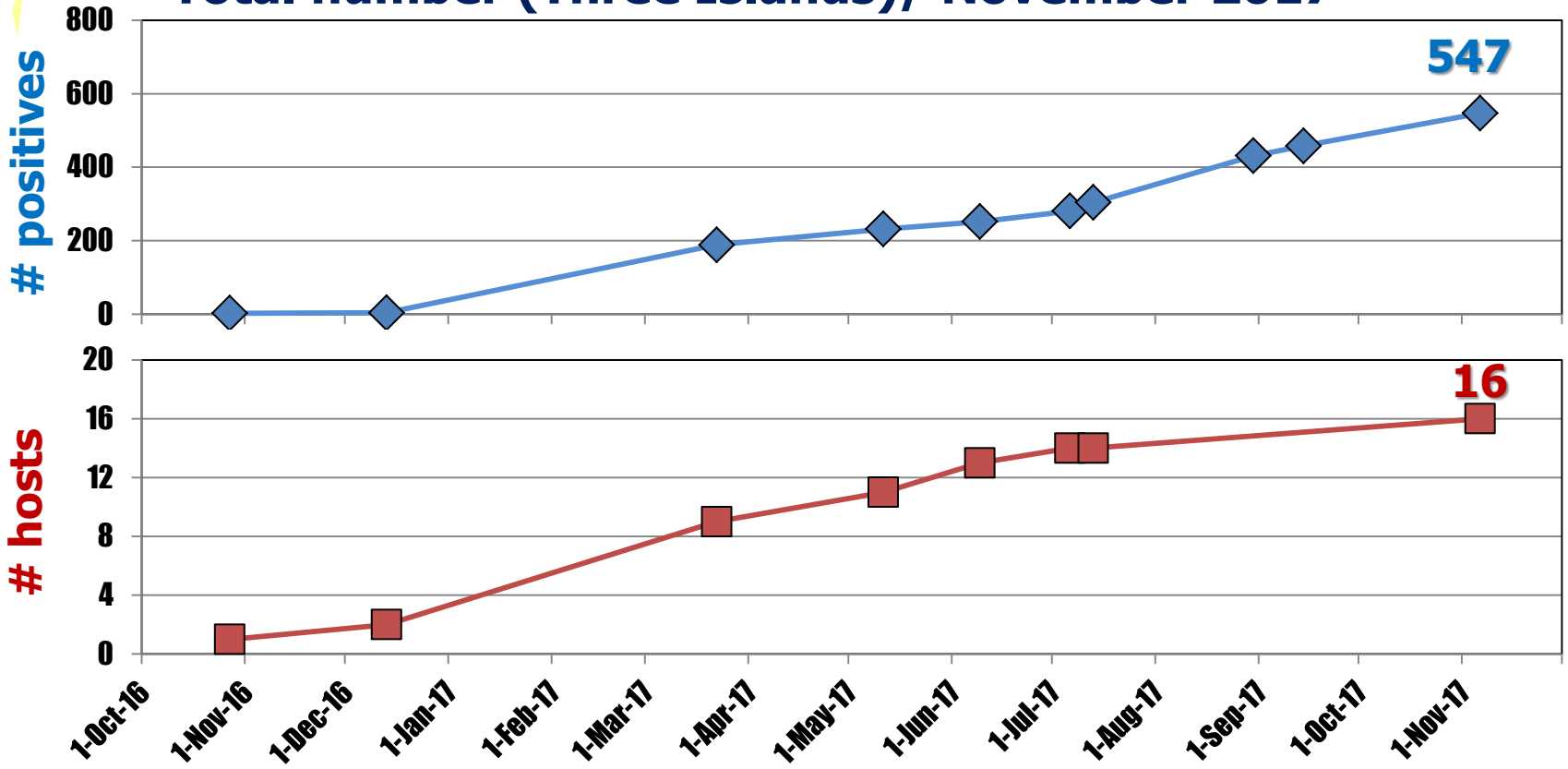
Buffer zone samples taken in the demarcated area

More Xf-positives appeared:

- **In different host**
- **In different islands**

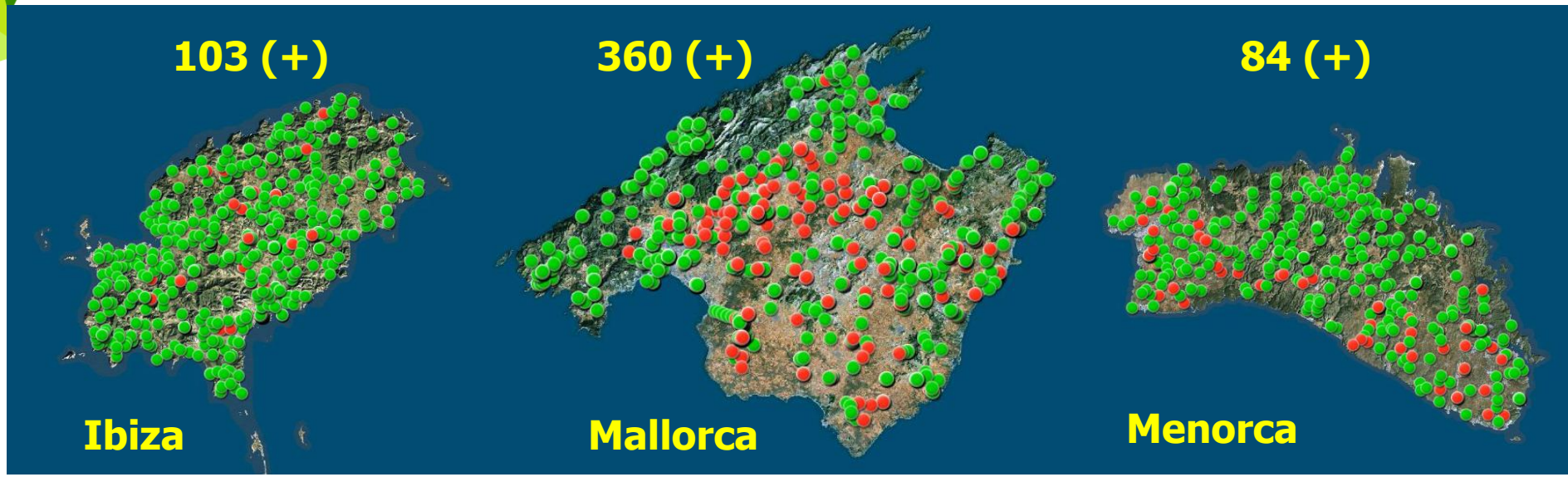
Situation of *Xylella fastidiosa* in the Balearic islands

Total number (Three Islands)/ November 2017



Situation of *Xylella fastidiosa* in the Balearic islands

➢ **Positives appeared spread throughout the three main islands**



ST	leuA	petC	malF	cysG	holC	nuoL	gltT	Subsp
1	1	1	1	1	1	1	1	*fastidiosa*
6*	3	3	3	3*	3	3	3	*multiplex*
7	3	3	3	7	3	3	3	*multiplex*
73*	7	6	8*	27*	10	16	8*	*pauca*

Pending new numbering for allele and ST assignation

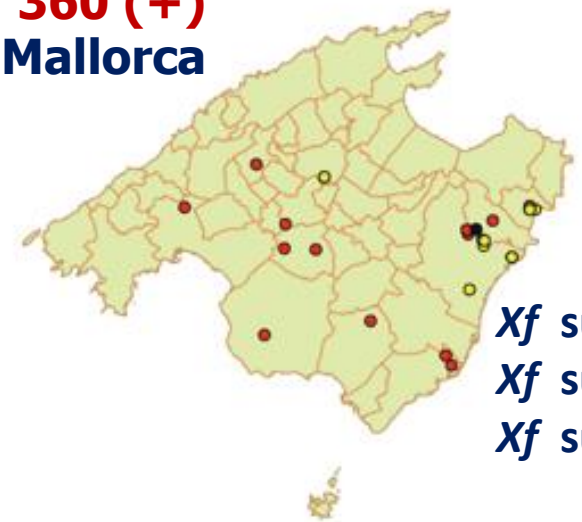
New alleles and ST described

Situation of *Xylella fastidiosa* in the Balearic islands

➢ Three subspecies of *Xylella fastidiosa* and four Sequence types (ST)

Olive 4%
Figs 5%
Grapes 13%
Oleaster 30%
Almond 39%

360 (+)
Mallorca



Xf subsp. *multiplex* ST6*
Xf subsp. *multiplex* ST7
Xf subsp. *fastidiosa* ST1

Olive 8%
Oleaster 76%

Menorca 103 (+)

Xf subsp. *multiplex* ST6*

84 (+)
Ibiza

Xf subsp. *pauca* ST80

Olive 33%
Oleaster 50%

Subspecie

- Multiplex ST 7*
- Fastidiosa ST 1
- Multiplex ST 6*
- Pauca ST 80

Total positives = 547 (Nov 2017)

Situation of *Xylella fastidiosa* in the Balearic islands

➢ **Host plants: 16 species of crops, ornamentals and natural vegetation**

Grapes

***fastidiosa* ST1**

Almond

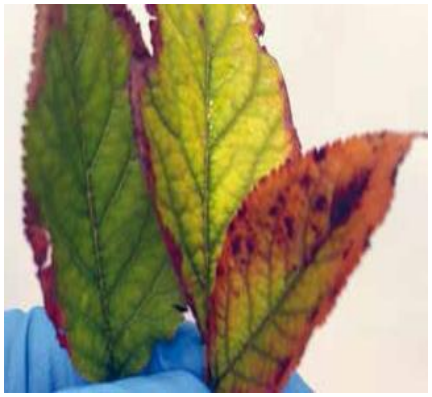
***fastidiosa* ST1**

Cherry

***fastidiosa* ST1**

Plum

Subsp. ?



Olive

***multiplex* ST6* / *pauca* ST80**

Oleaster

***multiplex* ST6* / *pauca* ST80**

Fig

multiplex* ST6

Walnut

Subsp. ?

Situation of *Xylella fastidiosa* in the Balearic islands

➢ **Host plants: 16 species of crops, ornamentals and natural vegetation**

Polygala myrtifolia

All ST 1, ST6*, ST7, ST80

Lavandula dentata

***multiplex* ST6* / *pauca* ST80**

Rosmarius officinalis

Subsp. *multiplex* ST6*

Cistus monspeliensis

Subsp. *fastidiosa* ST1

Fraxinus angustifolia

Subsp. *multiplex* ST6*

***Acacia* sp.**

***multiplex* ST6* / *pauca* ST80**

Nerium oleander

Subsp. ?

Rhamnus alaternus

Subsp. ?

Situation of *Xylella fastidiosa* in the Balearic islands

Olive (*Olea europea* var. *europaea*) ***Xylella fastidiosa*** **subsp. *multiplex***

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Situation of *Xylella fastidiosa* in the Balearic islands

Olive (*Olea europea* var. *europaea*) ***Xylella fastidiosa* subsp. *multiplex***

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Situation of *Xylella fastidiosa* in the Balearic islands

Olive (*Olea europea* var. *europaea*) ***Xylella fastidiosa* subsp. *multiplex***

Situation of *Xylella fastidiosa* in the Balearic islands

Almond (*Prunus dulcis*)

Xylella fastidiosa subsp. *fastidiosa*


Foto: Blanca B Landa


Foto: Blanca B Landa


Foto: Andreu Juan


Foto: Andreu Juan

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Situation of *Xylella fastidiosa* in the Balearic islands

Almond (*Prunus dulcis*) *Xylella fastidiosa* subsp. *fastidiosa*

Situation of *Xylella fastidiosa* in the Balearic islands

Grapes (*Vitis vinifera*)

Xylella fastidiosa subsp. *fastidiosa*

Situation of *Xylella fastidiosa* in the Balearic islands

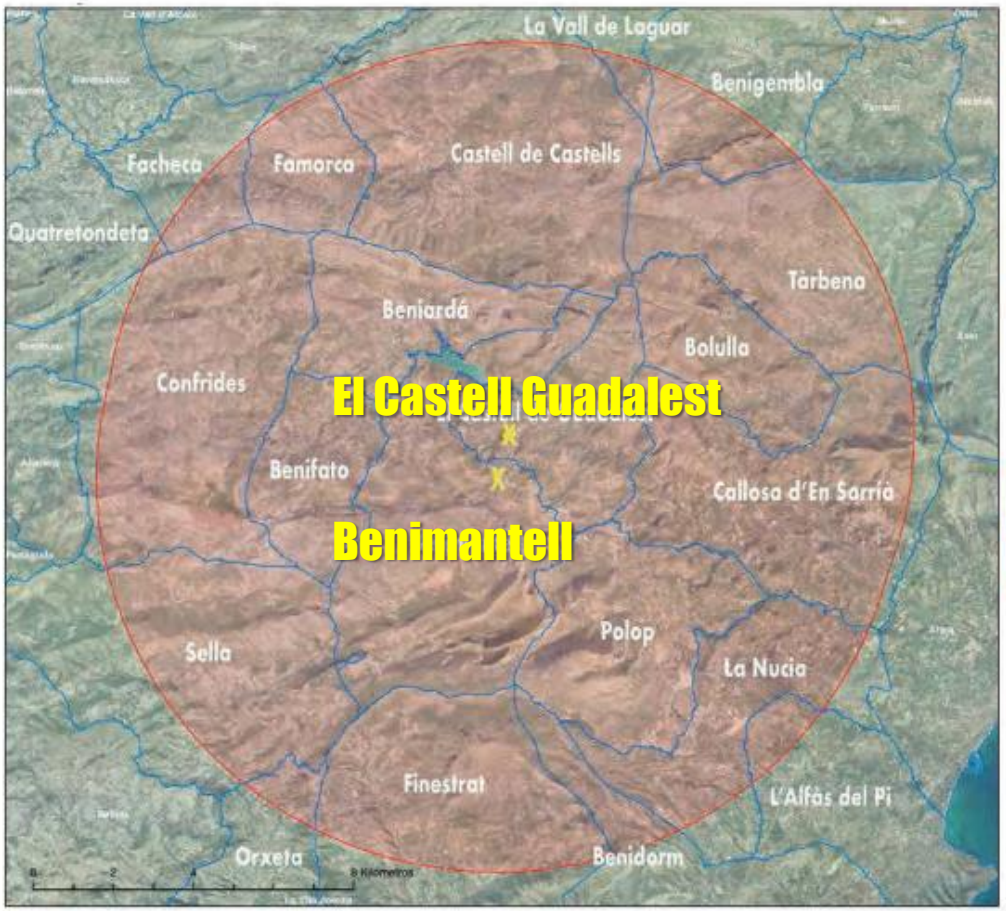
➢ **From middle 2017 a Contention Plan proposed: Eradication of positives**

About 60% of samples eliminated

Island	Number of Positives	Positive eliminated	TOTAL eliminated
Mallorca	360	171	2.263
Ibiza	103	88	318
Menorca	84	64	98
Formentera	0	0	1
TOTAL	547	323	2680

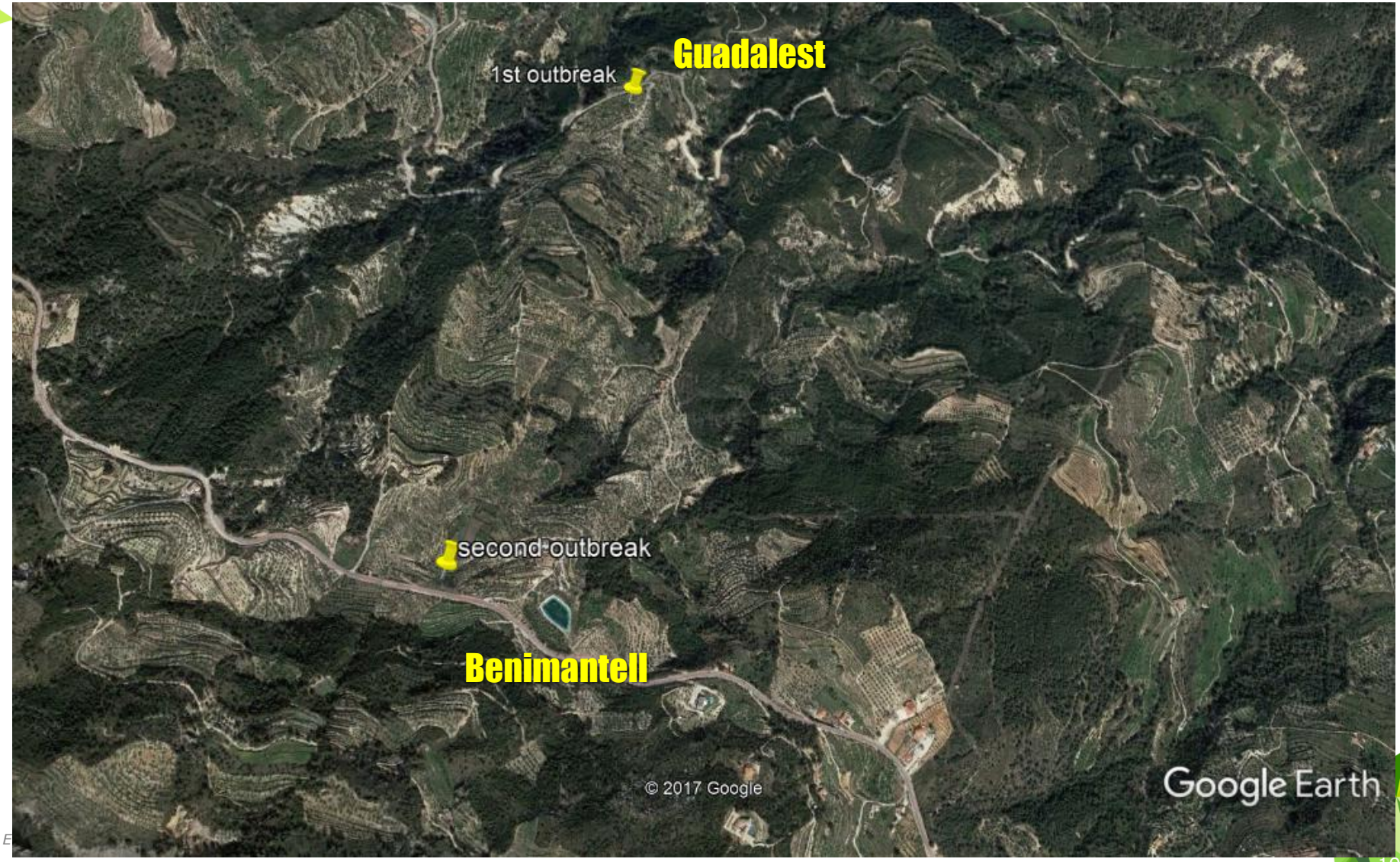
Situation of *Xylella fastidiosa* in mainland Spain (Alicante)

- 1st outbreak: 29 June 2017, 1 orchard
- 2nd Outbreak: 25 July 2017, 1 orchard (34.383 has. demarcated)



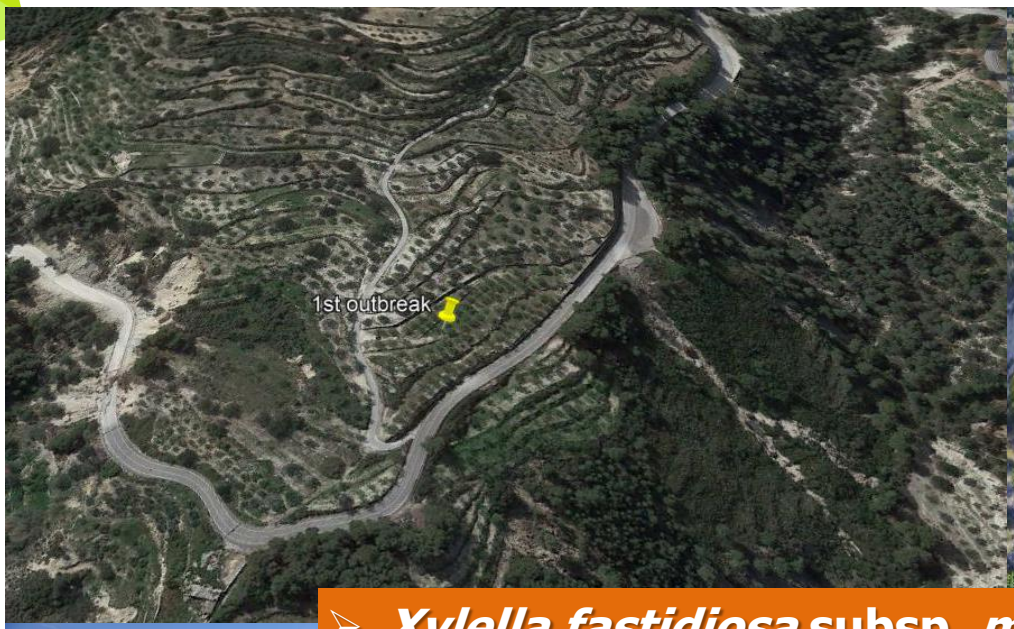
Situation of *Xylella fastidiosa* in mainland Spain (Alicante)

- 1st outbreak: 29 June 2017, 1 orchard; 2nd Outbreak: 25 July 2017, 1 orchard



Situation of *Xylella fastidiosa* in mainland Spain (Alicante)

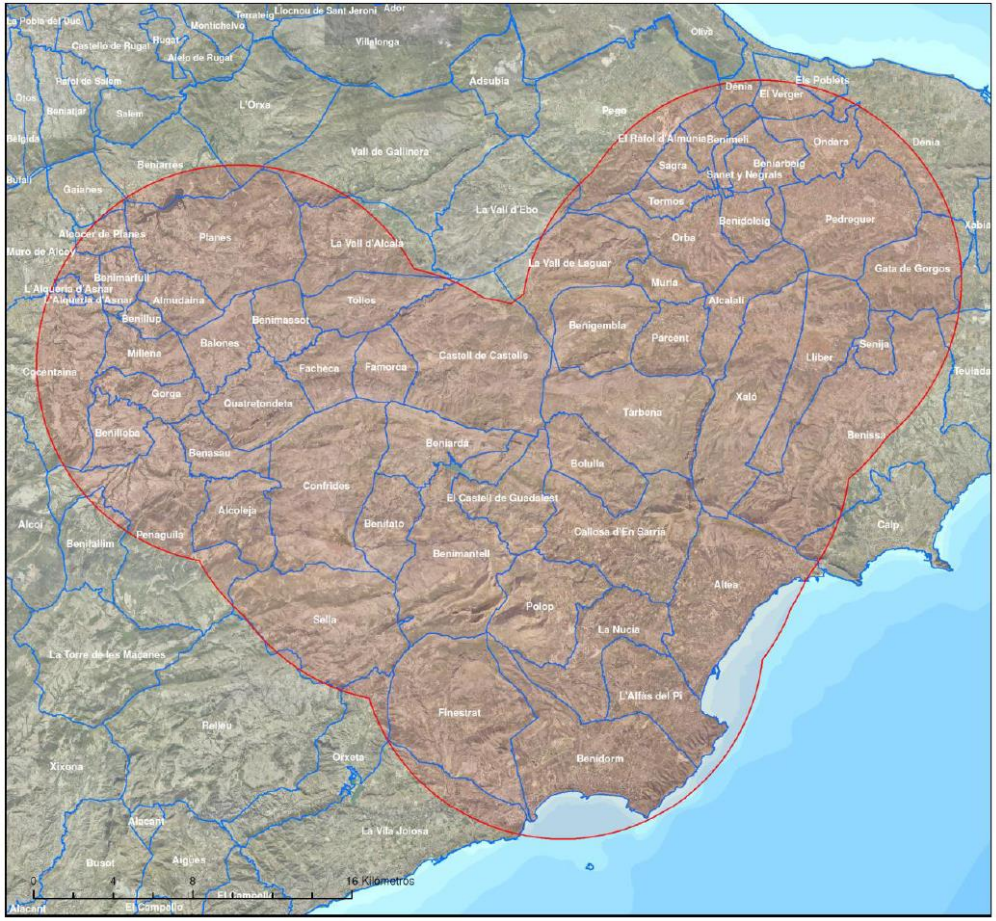
- 1st outbreak: 29 june 2017, 1 orchard; 2nd Outbreak: 25 July 2017, 1 orchard

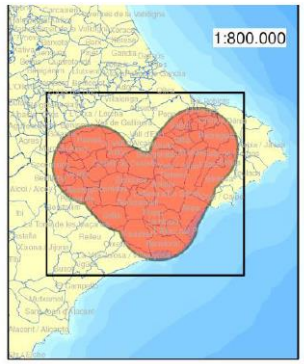


- *Xylella fastidiosa* subsp. *multiplex* ST6

Situation of *Xylella fastidiosa* in mainland Spain (Alicante)

- **3rd outbreak: 31 August 2017, 26 almond plots (110.945 has demarcated)**





Zona Demarcada

Conselleria d'Agricultura, Medi Ambient, Canvi Climàtic i Desenvolupament Rural
Dirección General de Agricultura, Ganadería y Pesca

PLAN DE CONTROL DE
Xylella fastidiosa
PROVINCIA DE ALICANTE

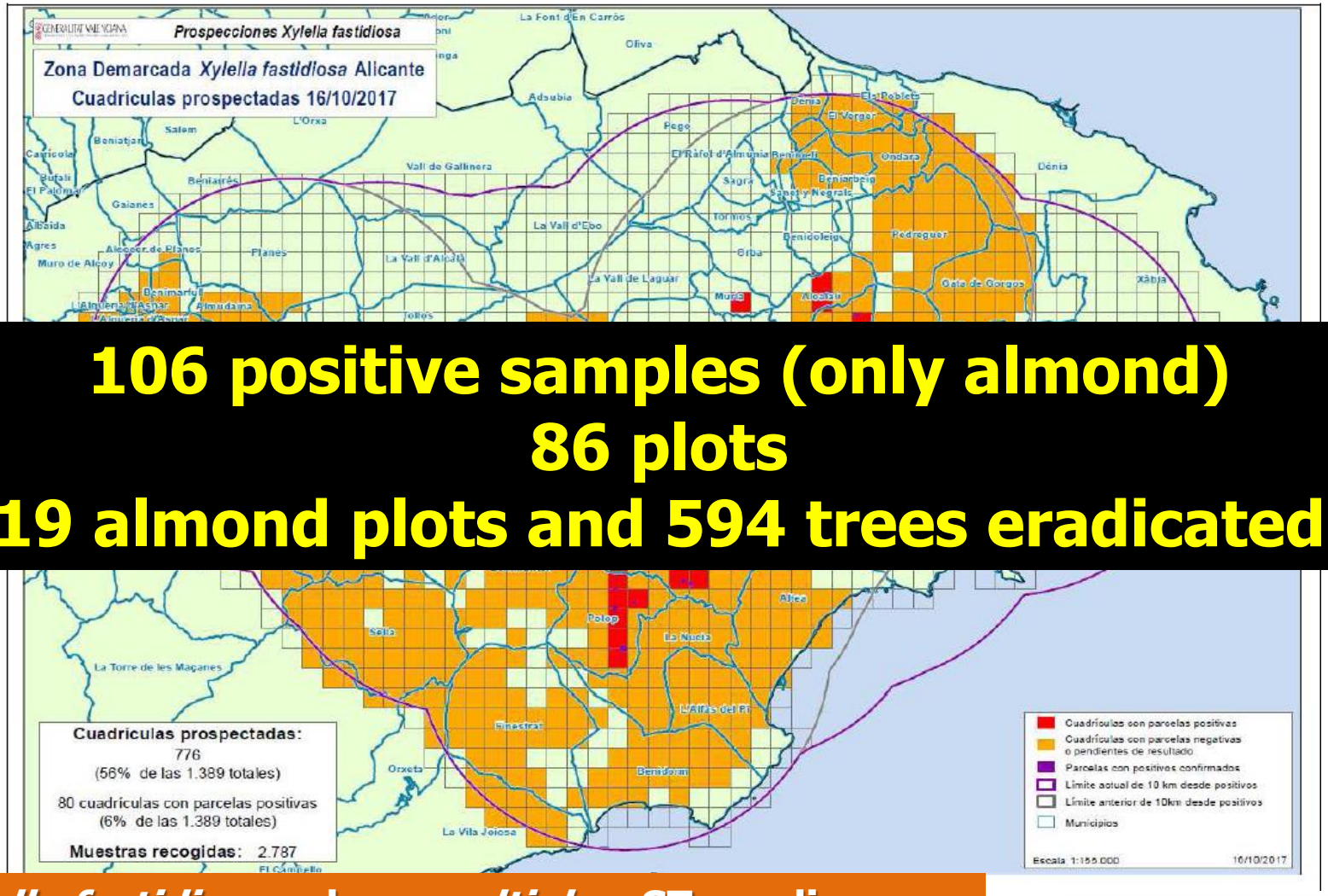
Zona Demarcada

Escala	Fecha
1:160.000	31/08/2017

- ***Xylella fastidiosa* subsp. *multiplex* ST pending**

Situation of *Xylella fastidiosa* in mainland Spain (Alicante)

- **4th outbreak: 20 October 58 almond plots (139.789 has. demarcated)**

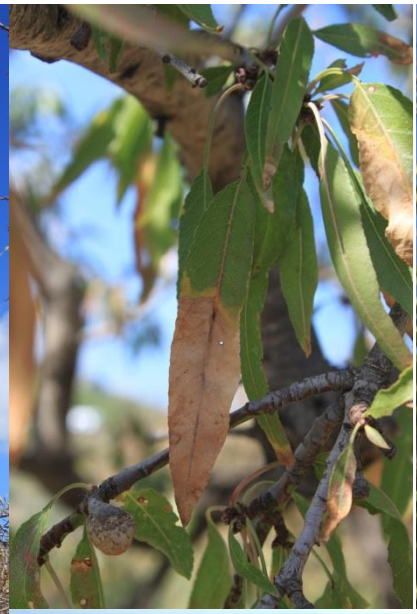


106 positive samples (only almond)
86 plots
19 almond plots and 594 trees eradicated

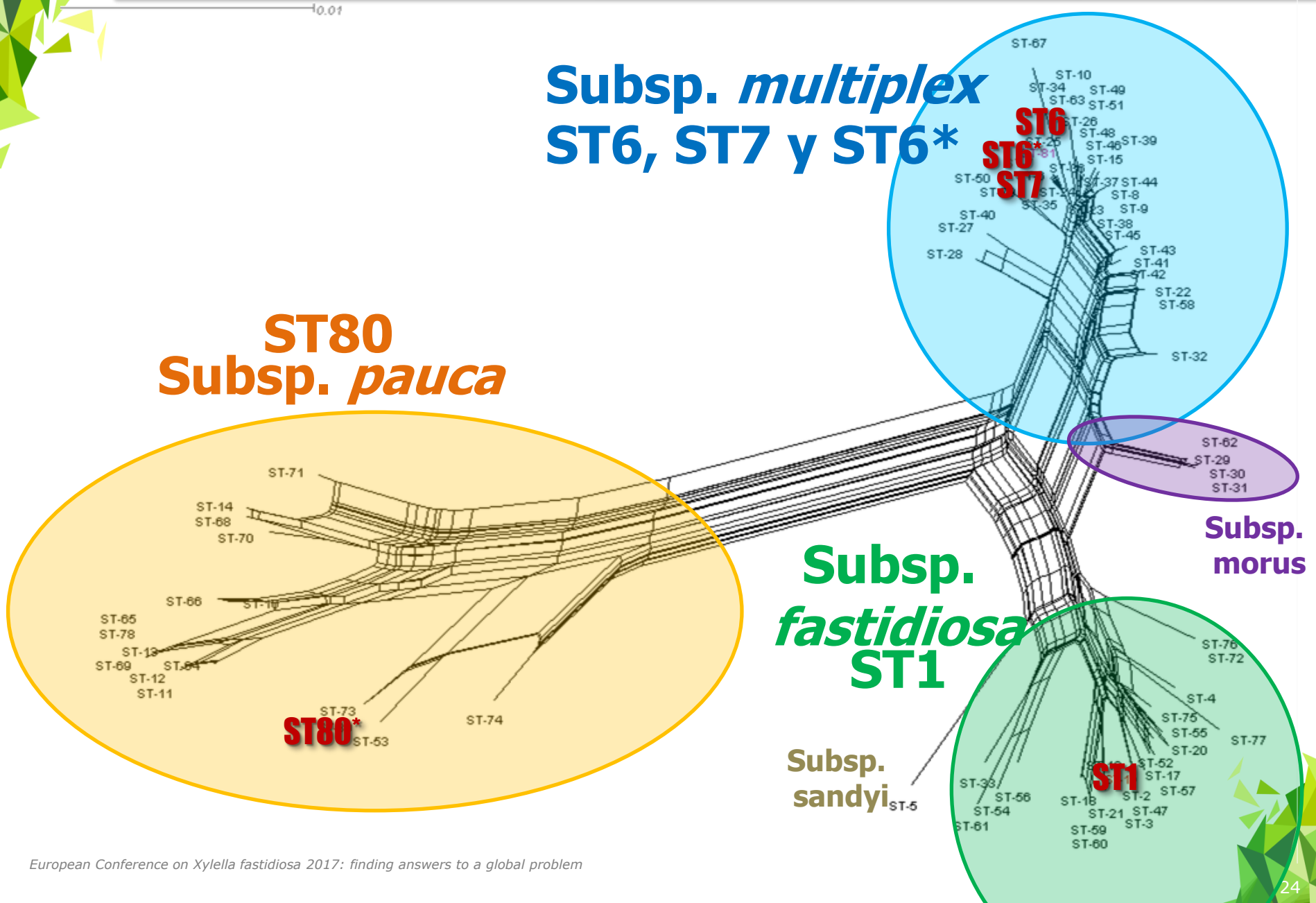
- ***Xylella fastidiosa* subsp. *multiplex* ST pending**

Situation of *Xylella fastidiosa* in mainland Spain (Alicante)

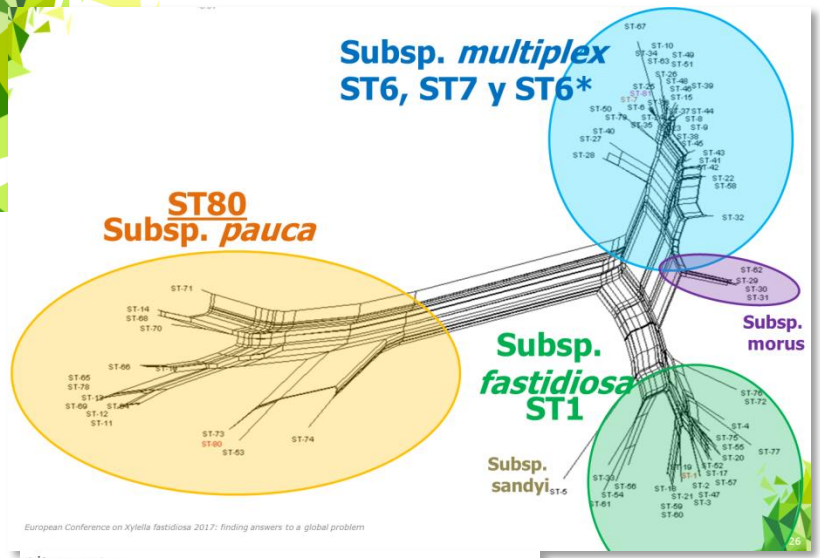
Almond (*Prunus dulcis*) *Xylella fastidiosa* subsp. *multiplex* ST 6



Current genetic diversity of *Xylella fastidiosa* in Spain

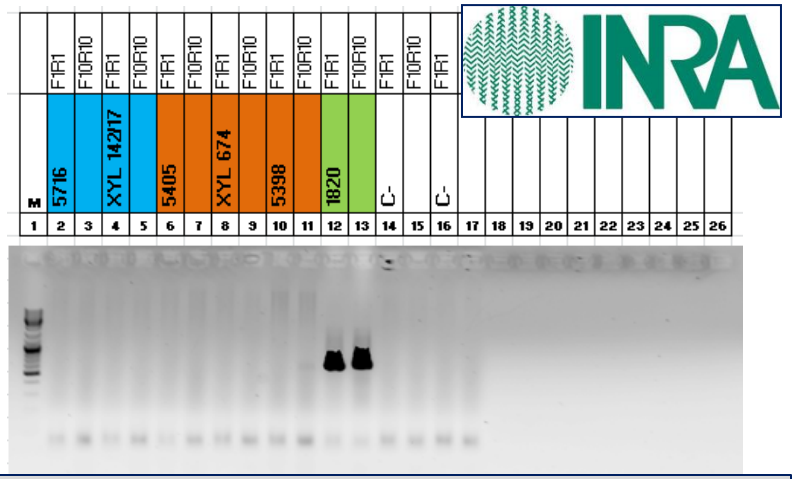


Current genetic diversity of *Xylella fastidiosa* in Spain

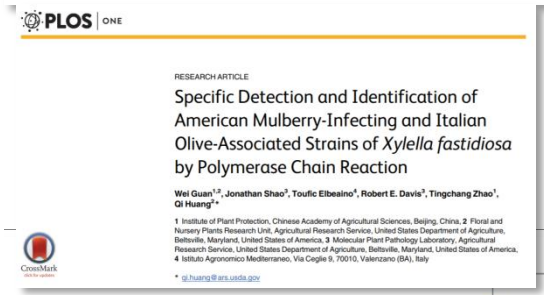


ST6≈ST6*

rpoD
pilU
acvB
fimA



Plasmid pXF-RIV5 involved in conjugation and DNA transfer among bacteria seems to be present in samples of subsp. *fastidiosa* ST1 but not in *multiplex* ST6/ST6* or *pauca* ST80


PLOS ONE

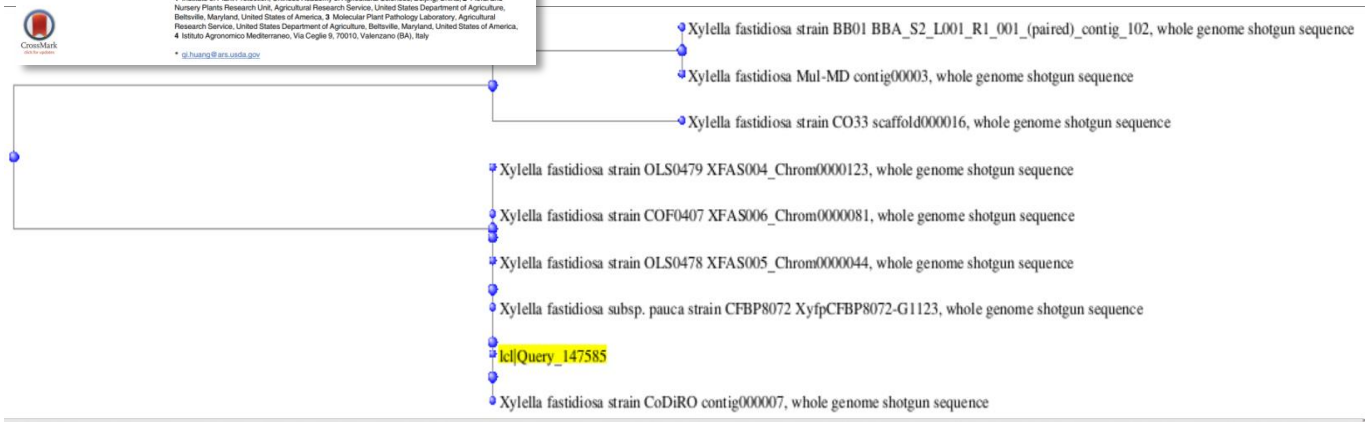
RESEARCH ARTICLE

Specific Detection and Identification of American Mulberry-Infecting and Italian Olive-Associated Strains of *Xylella fastidiosa* by Polymerase Chain Reaction

Wei Guan[1,2], Jonathan Shao[3], Toufic Elbeaino[4], Robert E. Davis[3], Tingchang Zhao[1], Qi Huang[2]*

1 Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, 2 Floral and Nursery Plants Research Unit, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland, United States of America, 3 Molecular Plant Pathology Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland, United States of America, 4 Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010, Valenzano (BA), Italy

* qi.huang@ars.usda.gov

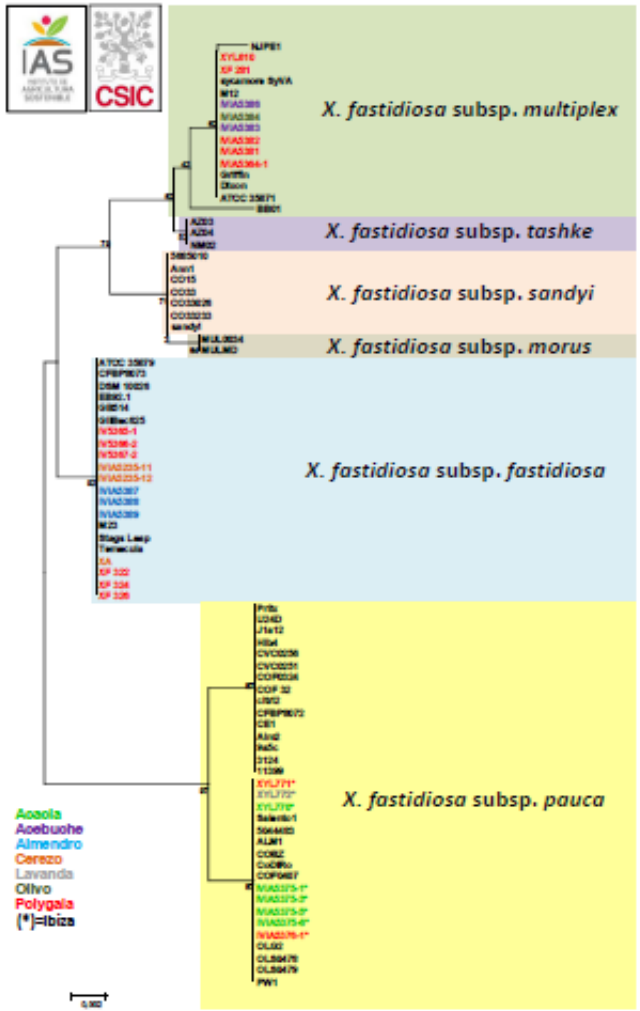


ST80 samples have the unique open reading frame sequence 'specific' for mulberry strains that also is present in other *pauca* strains

Current genetic diversity of *Xylella fastidiosa* in Spain

➢ Difficult to identify the subspecies/ST for several positive samples

rpoD (Minsavage *et al.*,1994)



- About 45% of samples identified to the subspecies/ST level using the MLST approach
- DNA used from plant extracts (inhibition, low bacteria titer or Ct values high: >30)
- Most samples were initially processed in winter or early spring and many plants have been eradicated
- More than 70% of samples could be identified now at the subspecies level if following recommendations of revised EPPO protocol
- Strain isolation success was initially low, but now better -> sequencing of genomes

Revision of the EPPO protocol


Bulletin OEPP/EPPO Bulletin (2016) **0** (0), 1–38 ISSN 0250-8052. DOI: 10.1111/epp.12327

European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

PM 7/24 (2)

Diagnostics
Diagnostic

PM 7/24 (2) *Xylella fastidiosa*

Situation of *Xylella fastidiosa* in Spain: Conclusions

- **New host plants of *X. fastidiosa* have been described for Europe after outbreaks in Balearic islands and Alicante**
  - ***Ficus carica, Fraxinus angustifolia, Juglans regia, Prunus domestica, Vitis vinifera***
  - **ST6 widespread in France (Corsica) but not detected in almond (1 case only) whereas is the only host infected in Alicante so far**
- **New subspecies and sequence types for Europe has been described**
  - **Subsp. *fastidiosa* ST1**
  - **Subsp. *multiplex* ST6* (A case in olive in California?)**
  - **Subsp. *pauca* ST80 (New for science)**
- **Several and independent introductions have occurred in Europe and Spain in the past and from different origins (probably EEUU and Central America)**
- **Sequencing of the genome of representative isolates can provide insights into their origin and biology**

Acknowledgements

POnTE
PestOrganisms
ThreateningEurope

XF
ACTORS

Euphresco