



Emergence of *Xylella fastidiosa* in Spain: current situation

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Process for official diagnosis of *Xylella fastidiosa* in Spain



**Official Plant Health
Laboratory from
Balearic islands**

**Autonomous community
region level**

**Official Plant Health
Laboratory from
Comunitat Valenciana**



**National Reference
Laboratory for
Phytopathogenic Bacteria**

National level

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



**Laboratory of Biology &
Ecology of Soil Microbiota
IAS-CSIC**

National level

- **Subspecies characterization**

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

European Conference on *Xylella fastidiosa* 2017: finding answers to a global problem



Situation of *Xylella fastidiosa* in the Balearic islands

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

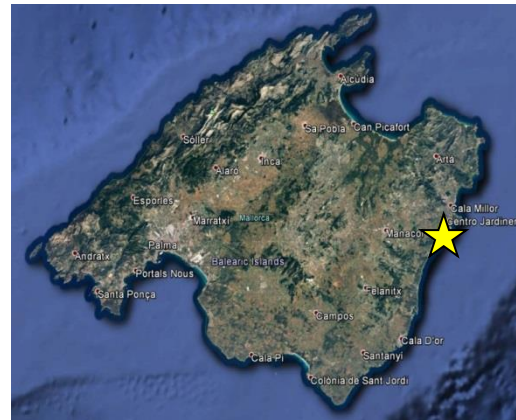
Official surveys
Decision EU 2015/789

Atypical symptoms



3 Cherry samples (+)

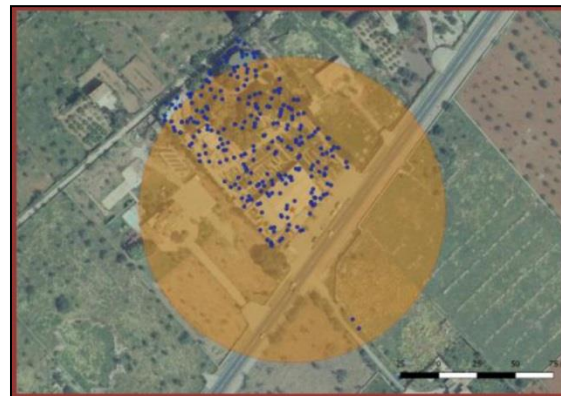
Typical symptoms



Infected zone



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

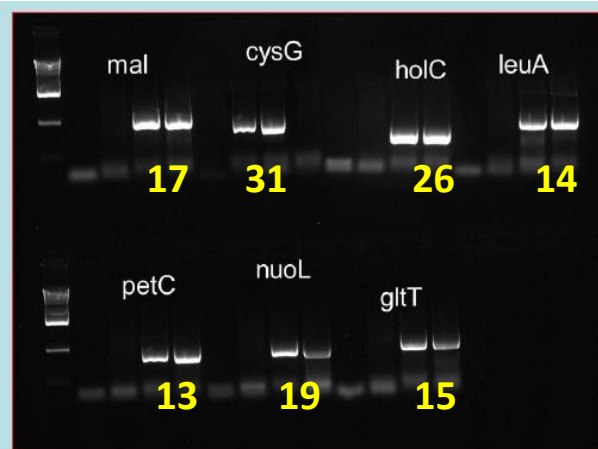


4 samples
Polygala myrtifolia
(+)

Situation of *Xylella fastidiosa* in the Balearic islands

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

MLST analysis



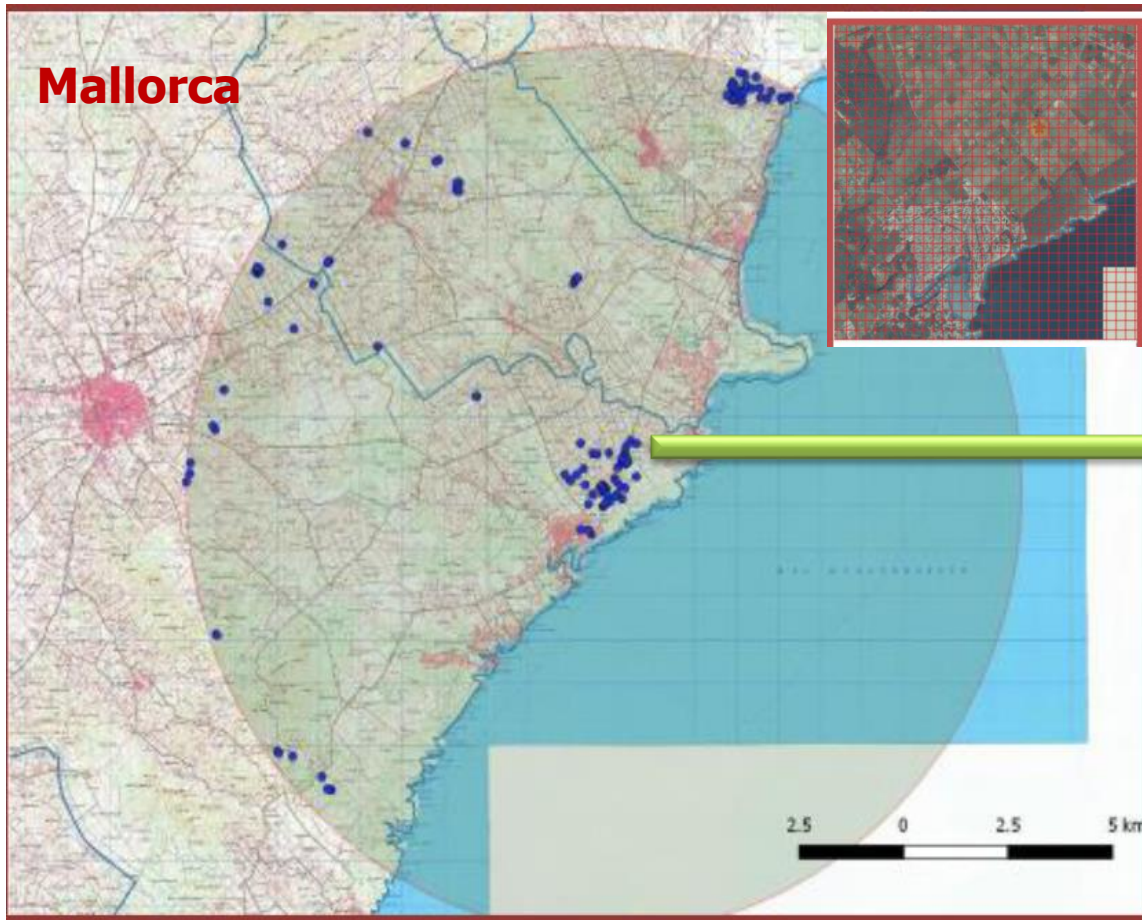
pilU and rpoD genes

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

Host	#plants	Subspecies/ST
Cherry	3	<i>Xf</i> subsp. <i>fastidiosa</i> ST1
<i>Polygala myrtifolia</i>	3	<i>Xf</i> subsp. <i>fastidiosa</i> ST1

Situation of *Xylella fastidiosa* in the Balearic islands

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



More Xf-positives appeared:

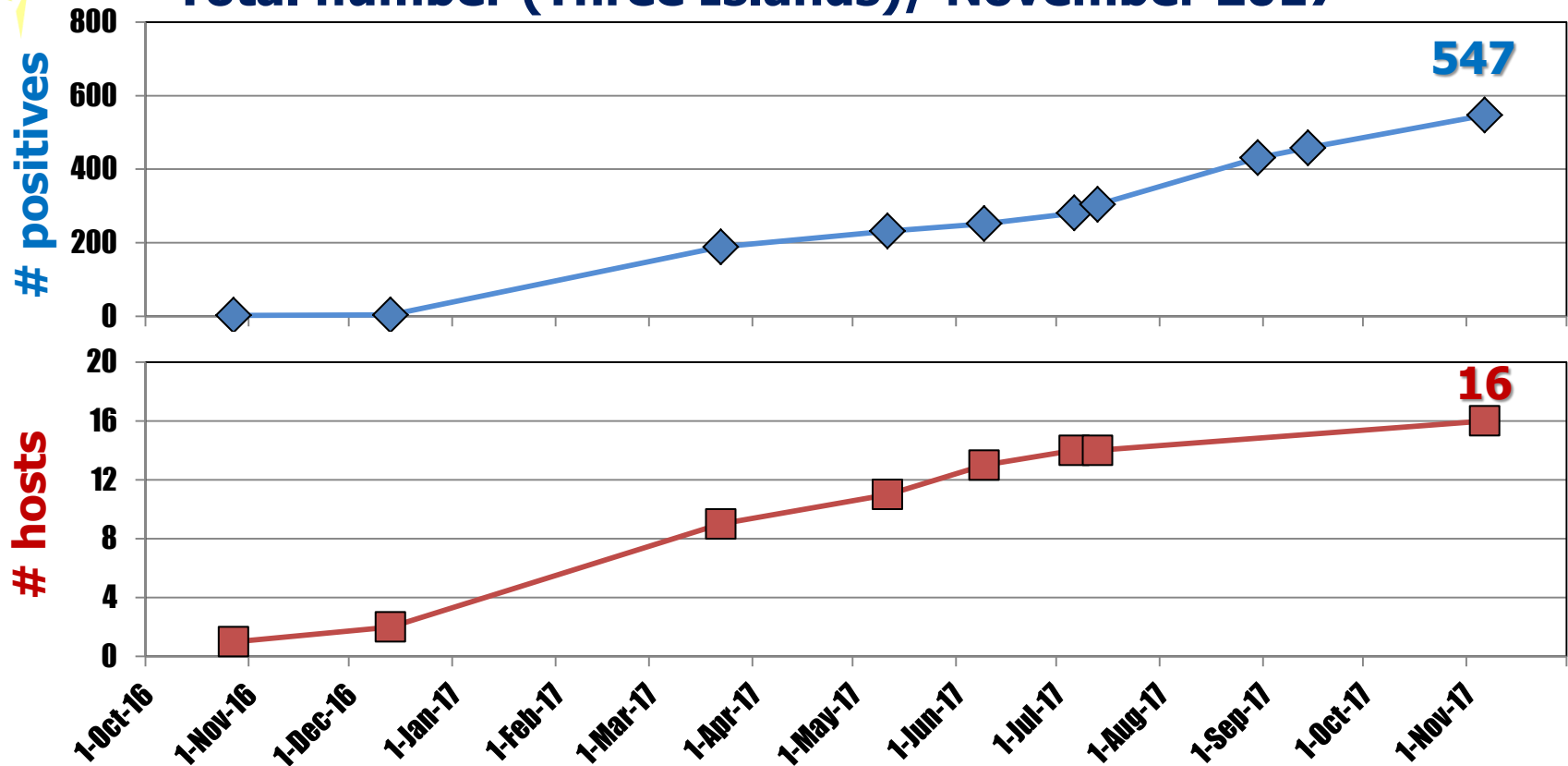
- In different host
- In different islands



Buffer zone samples taken in the demarcated area

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

103 (+)

360 (+)

84 (+)

Ibiza

Mallorca

Menorca

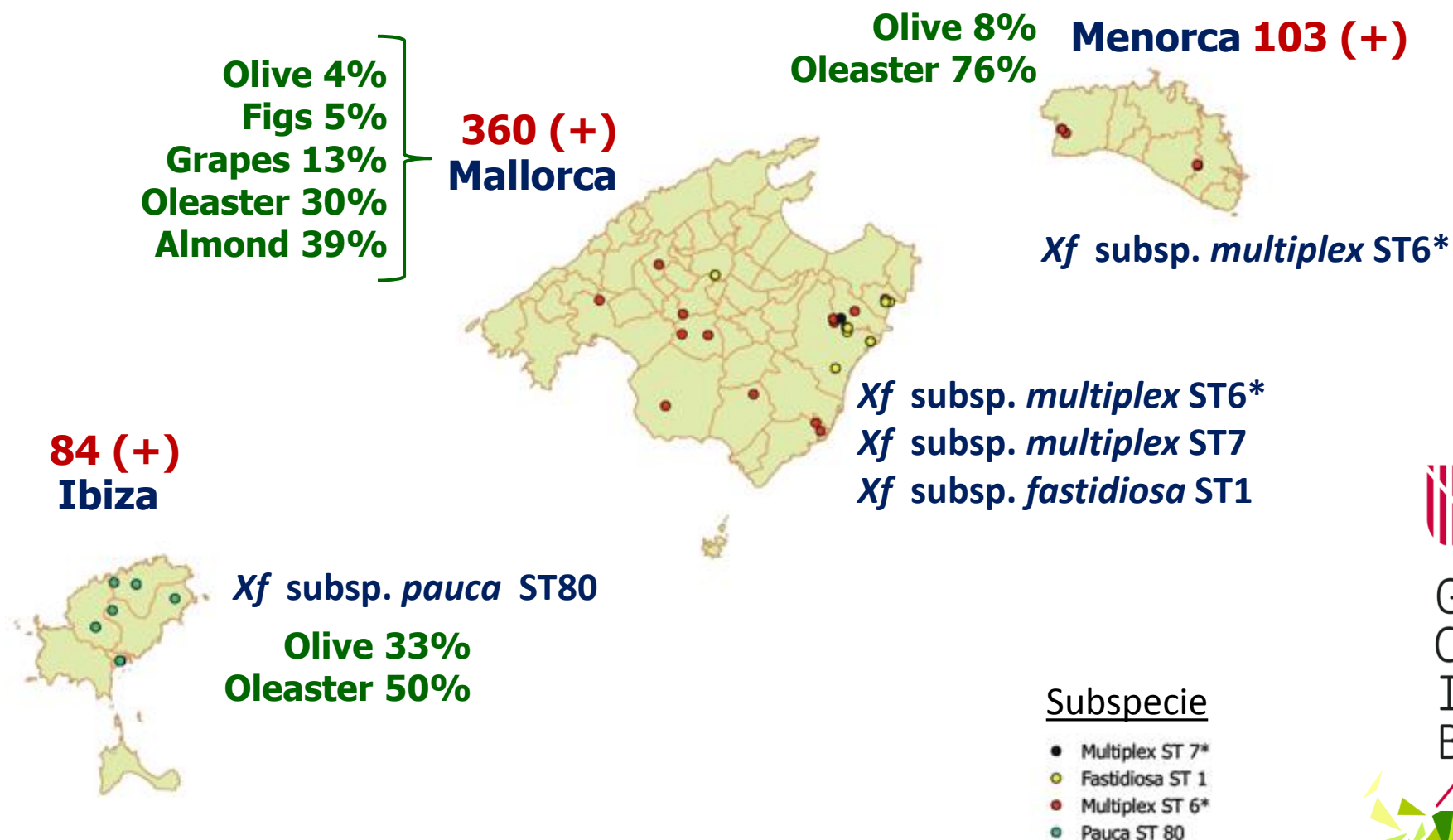
ST	leuA	petC	malF	cysG	holC	nuoL	gltT	Subsp
1	1	1	1	1	1	1	1	<i>fastidiosa</i>
6*	3	3	3	3*	3	3	3	<i>multiplex</i>
7	3	3	3	7	3	3	3	<i>multiplex</i>
73*	7	6	8*	27*	10	16	8*	<i>pauca</i>

Pending new
numbering for allele
and ST assignment

New alleles and
ST described

Situation of *Xylella fastidiosa* in the Balearic islands

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



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



Rosmarinus 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 europaea* var. *europaea*) *Xylella fastidiosa* subsp. *multiplex*



GOIB

Situation of *Xylella fastidiosa* in the Balearic islands

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



GOIB

Situation of *Xylella fastidiosa* in the Balearic islands

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



GOIB

Situation of *Xylella fastidiosa* in the Balearic islands

Almond (*Prunus dulcis*)

Xylella fastidiosa subsp. *fastidiosa*



GOIB

Situation of *Xylella fastidiosa* in the Balearic islands

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



GOIB



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

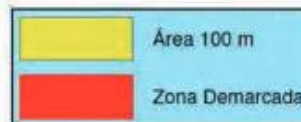
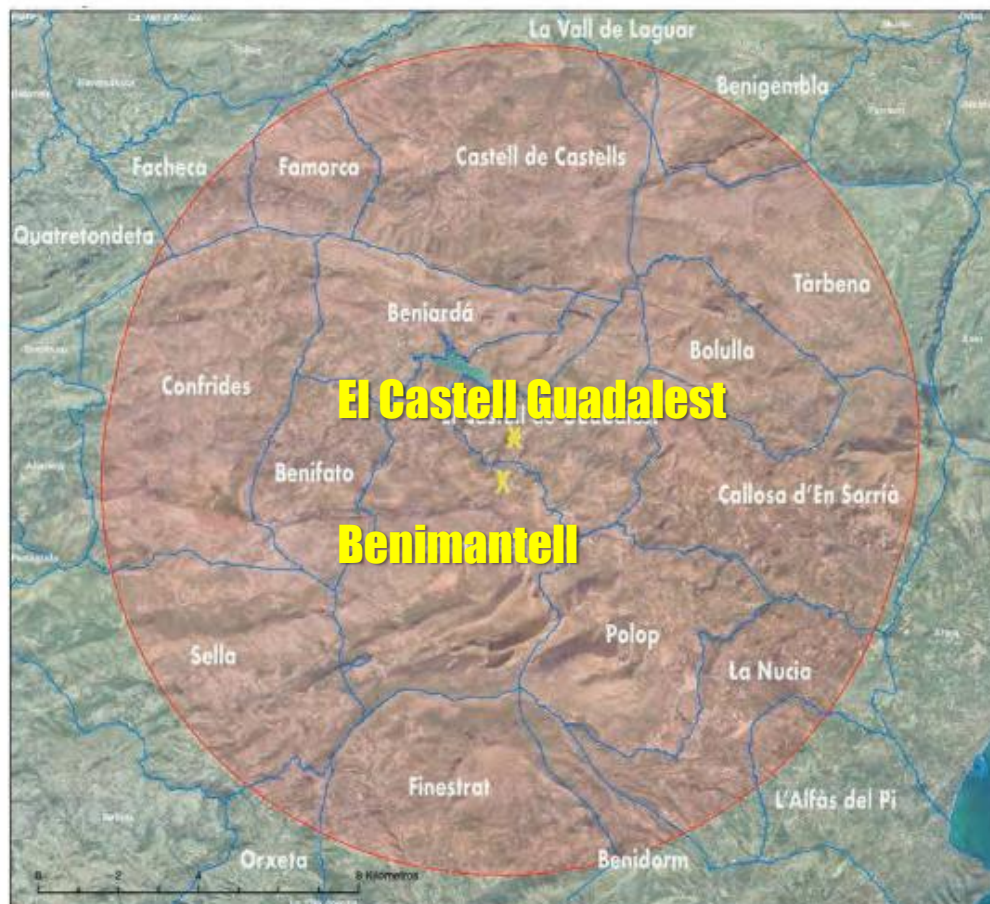
Island	Number of Positives	Positive eliminated	TOTAL eliminated
Mallorca	360	171	2.263
Ibiza	103	88	318
Menorca	84	64	98
Formentera	0	0	1
TOTAL	547	323	2680



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

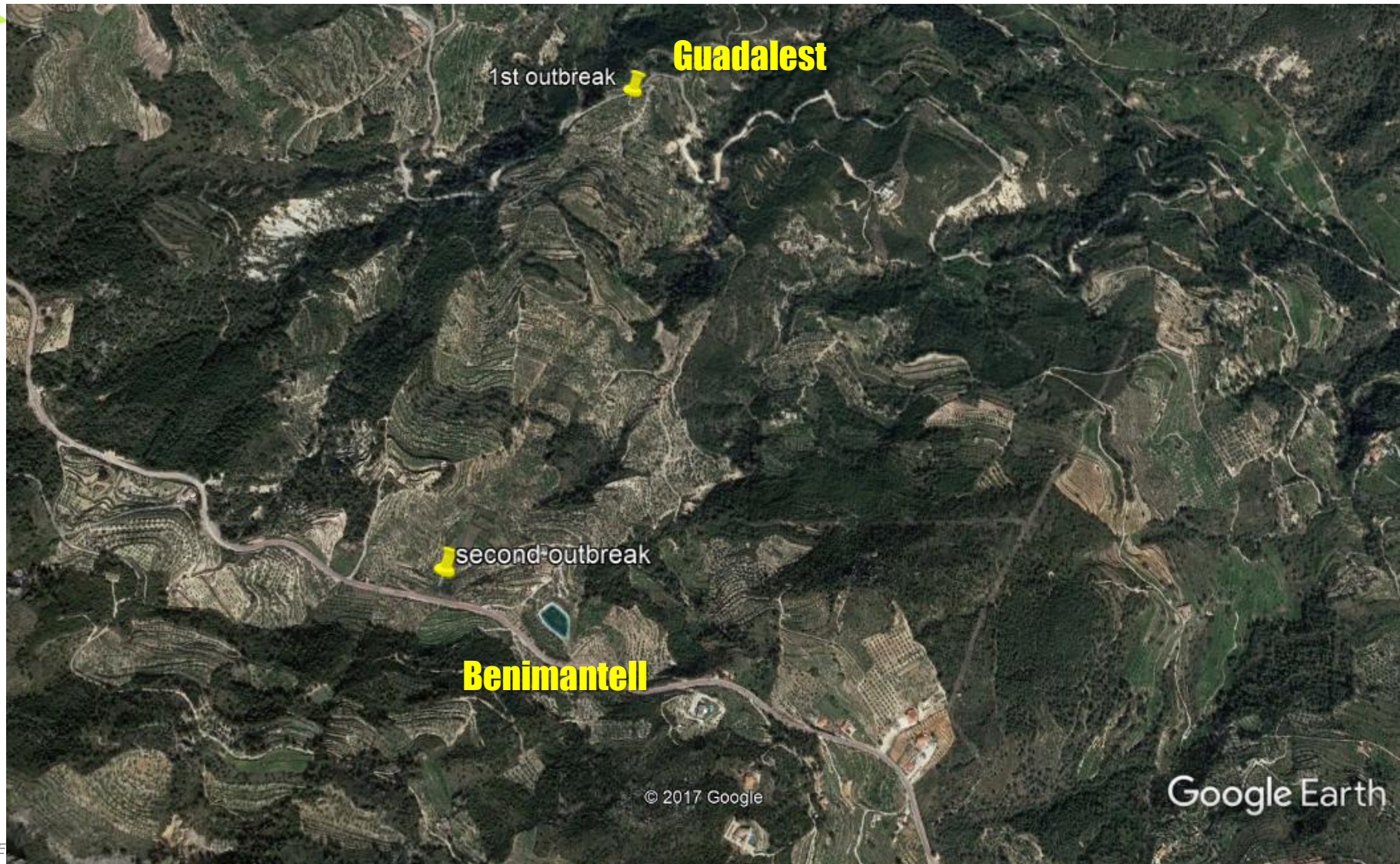
- 1st outbreak: 29 June 2017, 1 orchard
- 2nd Outbreak: 25 July 2017, 1 orchard

(34.383 has. demarcated)



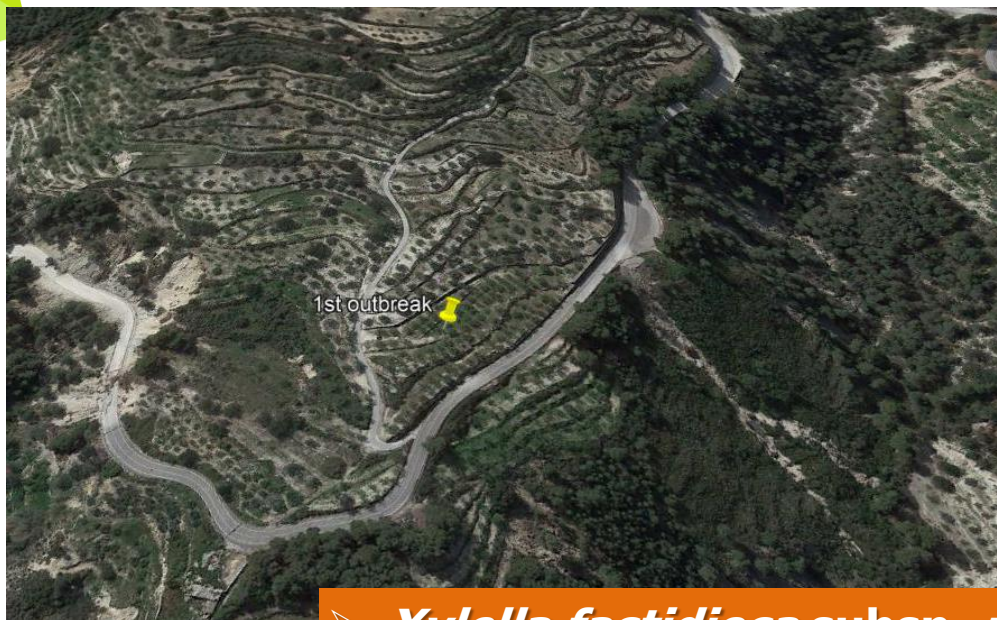
Situation of *Xylella fastidiosa* in mainland Spain (Alicante)

➤ **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

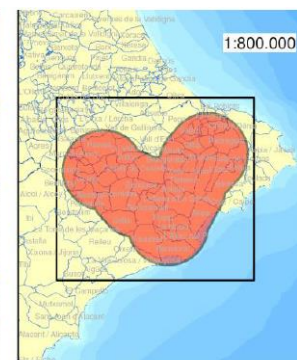


➤ *Xylella fastidiosa* subsp. *multiplex* ST6



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 Direcció General de Agricultura, Ganaderia y Pesca		
PLAN DE CONTROL DE <i>Xylella fastidiosa</i> PROVINCIA DE ALICANTE		
Zona Demarcada		
Escala	Fecha	
1:160.000	31/08/2017	

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

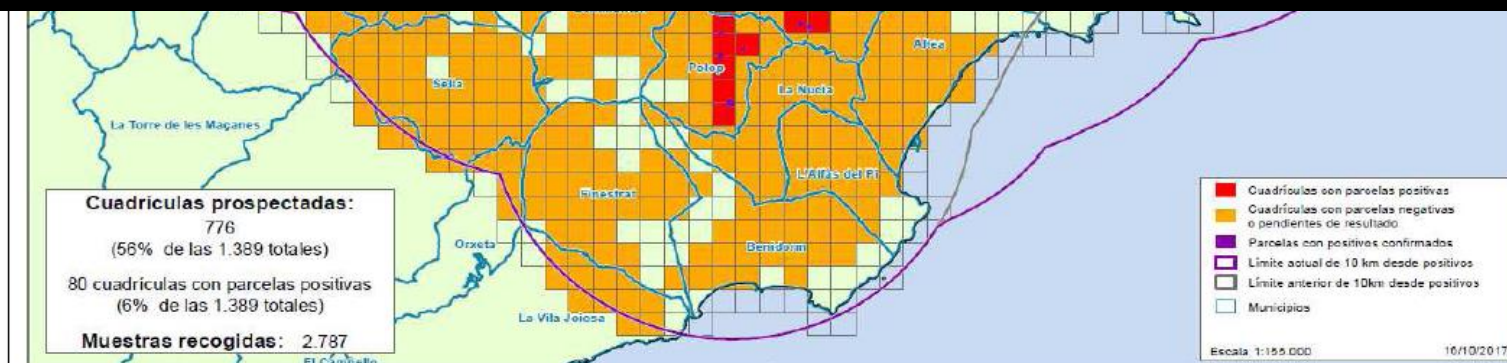
European Conference on *Xylella fastidiosa* 2017: finding answers to a global problem

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

10.01

Subsp. *multiplex*
ST6, ST7 y ST6*

ST80
Subsp. *pauca*

Subsp. *fastidiosa*
ST1

Subsp. *sandyi*

Subsp. *morus*

ST80*

ST1

Current genetic diversity of *Xylella fastidiosa* in Spain

Subsp. *multiplex*
ST6, ST7 y ST6*

ST80
Subsp. *pauca*

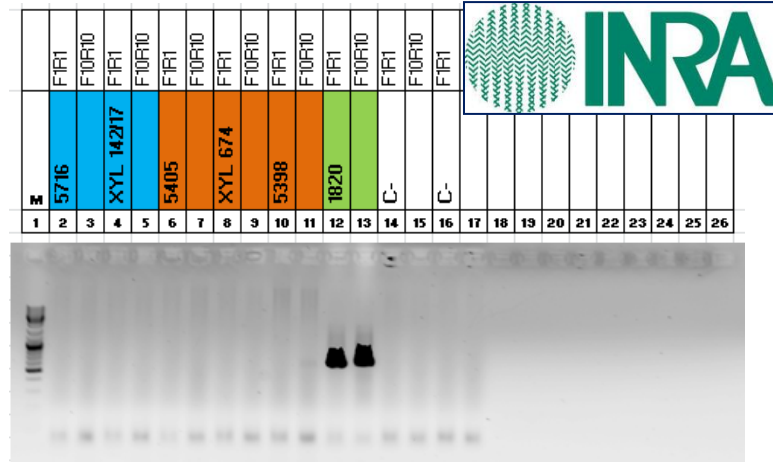
Subsp.
fastidiosa
ST1

Subsp.
*sandyi*_{ST5}

Subsp.
morus

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

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

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

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Xylella fastidiosa strain BB01 BBA_S2_L001_R1_001 (paired)_contig_102, whole genome shotgun sequence

Xylella fastidiosa Mul-MD contig00003, whole genome shotgun sequence

Xylella fastidiosa strain CO33 scaffold000016, whole genome shotgun sequence

Xylella fastidiosa strain OLS0479 XFAS004_Chrom0000123, whole genome shotgun sequence

Xylella fastidiosa strain COF0407 XFAS006_Chrom0000081, whole genome shotgun sequence

Xylella fastidiosa strain OLS0478 XFAS005_Chrom0000044, whole genome shotgun sequence

Xylella fastidiosa subsp. pauca strain CFBP8072 XyfpCFBP8072-G1123, whole genome shotgun sequence

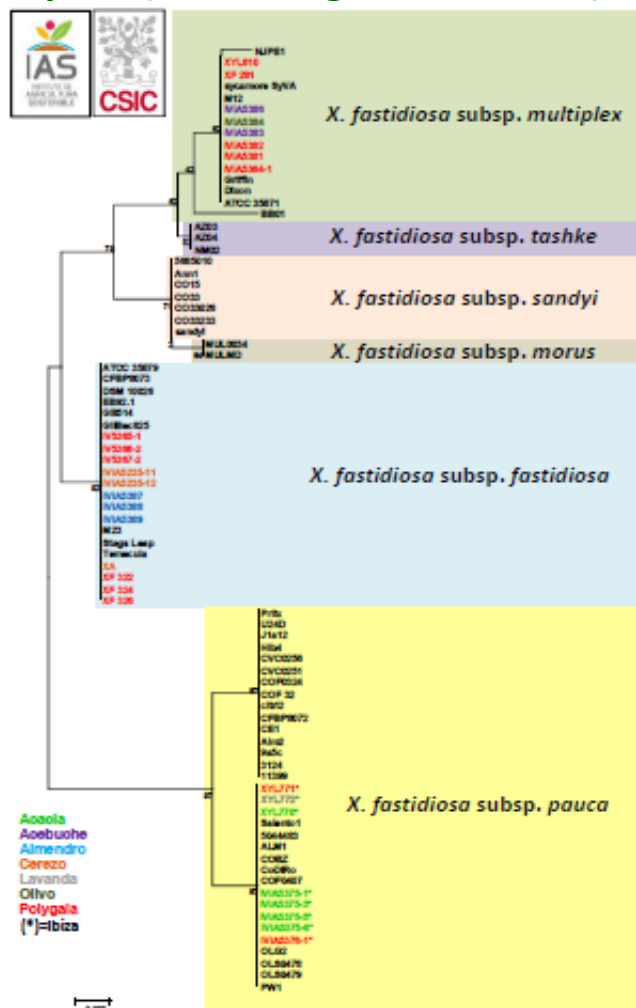
klcQuery_147585

Xylella fastidiosa strain CoDiRo contig000007, whole genome shotgun sequence

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 (2018) 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



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