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

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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**

*Olive Quick decline Syndrome*
Genetic diversity of *Xylella fastidiosa* in Europe

- **4 subpecies**
- **9 STs**
- **Most detections-outbreaks are from** *Xf multiplex*
- *Xf multiplex* accounts for the larger number of susceptible plant species (more than 75%)

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

Total host plants = 85
Situation of *Xylella fastidiosa* in Spain

**Balearic Islands**

1st notification
October 2016
Under containment
14 December 2017
843 positives (Feb. 2019)

**Ibiza**

228 (+)
Subsp. *pauca* ST80

**Mallorca**

476 (+)
Subsp. *fastidiosa* ST1
Subsp. *multiplex* ST7, ST81

**Menorca**

139 (+)
Subsp. *multiplex* ST81

**Alicante**

1st notification
June 2017
Under eradication

Subsp. *multiplex* ST6
Demarcated área: > 138,000 ha
> 1500 positives in 74 Municipalities
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. ?

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2nd European Conference on Xylella fastidiosa 2019
Situation of *Xylella fastidiosa* in the Balearic Islands

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

- *Lavandula dentata* multiplexST81/paucaST80
- *Rosmarinus officinalis* multiplexST81
- *Calicotome spinosa* fastidiosaST1
- *Cistus monspeliensis* fastidiosaST1
- *Cistus albidus* multiplexST81/paucaST80
- *Fraxinus angustifolia* multiplexST81
- *Acacia sp.* multiplexST81/paucaST80
- *Rhamnus alaternus* fastidiosaST1
- *Genista lucida* fastidiosaST1
- *Teucrium capitatum* fastidiosaST1
- *Lavandula angustifolia* multiplex ST81
Situation of Xylella fastidiosa in Alicante

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

X. fastidiosa subsp. multiplex * ST6**

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

Host plants:
- Prunus dulcis**
- Prunus armeniaca**
- Prunus domestica**
- Helichrysum italicum*
- Polygala myrtifolia**
- Rosmarinus officinalis**
- Rhamnus alaternus
- Phagnalon saxatile**
- Calicotome spinosa
- Lavandula dentata
- Lavandula latifolia
- Helichrysum stoechas
Host plants of *Xylella fastidiosa* subsp. *multiplex* in Spain

<table>
<thead>
<tr>
<th>Region</th>
<th>N</th>
<th>Host plants</th>
</tr>
</thead>
</table>
| 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* |
| Mallorca & 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

2nd European Conference on Xylella fastidiosa 2019
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)
- Assignment 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

- 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

**Subsp. pauca**

**Subsp. multiplex**

**Subsp. morus**

**Subsp. fastidiosa**

- 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

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

- 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

**ML phylogenetic tree (rooted)**

- 92% - 97% likelihood of Southeastern USA origin at node
- 51% - 69% likelihood of Southeastern USA origin at node

**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)

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

Occurrence of plasmids pXF64-Hb_ESVL and pUCLA-ESVL associated with infections caused by Xylella fastidiosa subsp. multiplex ST6 in the Demarcated Area of Alicante, Spain

**INTRODUCTION**

Apical inoculation using multiple ST6 was identified in Valencia Governor in June of 2017. In terms of 2018, the first appearance was reported in 21 municipalities within the Mediterranean area of Alicante province (Pineapple Cultivation) (Garlic & Tomato 2017). The current situation now (2019) is critical, marked by the emergence of a new form called Xylella fastidiosa del Caron and affects more than 73 municipalities, 75 from Alicante and 9 from Valencia provinces.

**MATERIAL AND METHODS**

Symptoms (10 plants) were monitored for the Xylella fastidiosa in Vivo model (10 plants).

**RESULTS**

- pUF64-Hb plasmid detected (7 plants).
- pUCLA-ESVL plasmid detected (3 plants).
- no association detected (8 plants).

**CONCLUSIONS**

- The inoculation of the plasmid is the main route of transmission of the pathogen to new hosts.

**REFERENCES**

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

**Session 8: Biology and Pathogenicity II**

Phenotypic characterization of two Spanish strains of *Xylella fastidiosa* subsp. *multiplex* ST6 differing in plasmid content
Evolutionary relationships between European/American *X.fastidiosa* populations

**ML phylogenetic tree**
Based on 5630 core SNPs

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

<table>
<thead>
<tr>
<th>Origin</th>
<th>Shared SNPs within the cluster</th>
<th>Differentiating SNPs within the cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alicante</td>
<td>79</td>
<td>10</td>
</tr>
<tr>
<td>Balearic Islands</td>
<td>41</td>
<td>3</td>
</tr>
<tr>
<td>Tuscany</td>
<td>129</td>
<td>1</td>
</tr>
</tbody>
</table>

**Nucleotide diversity (¶)**

<table>
<thead>
<tr>
<th></th>
<th>California</th>
<th>Europe</th>
<th>Southeastern USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>59.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td>60.06</td>
<td>54.27</td>
<td></td>
</tr>
<tr>
<td>Southeastern USA</td>
<td>289.21</td>
<td>293.81</td>
<td>354.40</td>
</tr>
</tbody>
</table>

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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)

<table>
<thead>
<tr>
<th>Olive Genotypes</th>
<th>Picual, Hojiblanca y Arbequina</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strains subsp. multiplex</strong></td>
<td>IVIA5901 &amp; ESVL (ST6) &amp; 1966 (ST81)</td>
</tr>
<tr>
<td><strong>Strains subsp. pauca</strong></td>
<td>De Donno (ST53) &amp; 1961 (ST80)</td>
</tr>
<tr>
<td><strong>Experiments</strong></td>
<td>2 experiments: 180 plant inoculated</td>
</tr>
</tbody>
</table>

**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.
Pathogenicity experiments in olive (Spain)

*X. fastidiosa pauca* (ST53, ST80) and *X. fastidiosa multiplex* (ST6, ST81)

No symptoms developed after seven months.
Pathogenicity experiments in olive (Spain)

*X. fastidiosa pauca* (ST53, ST80) and *X. fastidiosa multiplex* (ST6, ST81)

### Spectral signature (450-800 nm)

- **Reflectance**
- **Chlorophyll index**
  - Arbequina, Hojiblanca, Picual
- **Flavonol index**
  - Arbequina, Hojiblanca, Picual
- **Nitrogen index**
  - Arbequina, Hojiblanca, Picual

### Spectral signature (900-1700 nm)

- **Reflectance**
- **Nitrogen Balance Index**
  - 1st month, 6th month
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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