Rethinking the *Xylella fastidiosa* scenario in the Balearic Islands: what epidemiological, phylogenetic and dendrochronological data tell us

Moralejo E¹, Olmo D², M Gomila³, Nieto A², Montesinos M¹, Borràs D², Andreu J², Landa B B⁴

¹Tragsa, Empresa de Transformación Agraria, Delegación de Baleares, 07005 Palma de Mallorca, Spain.
²Serveis de Millora Agrària, Govern Balear, 07009, Palma de Mallorca, Spain.
³Microbiology (Biology Department), University of the Balearic Islands, 07122 Palma de Mallorca, Spain.
⁴Instituto de Agricultura Sostenible, CSIC, Córdoba, Spain.
1. Current situation in the Balearic Islands

- More than 7,250 samples analysed since 2016
- *Xylella fastidiosa* in 21 hosts
- Three subspecies present: *pauca*, *multiplex* and *fastidiosa*
- Four sequence type (ST): ST1 (subsp. *fastidiosa*), ST7 (*multiplex*), ST80 (*pauca*) and ST81 (*multiplex*)
1. Current situation in the Balearic Islands

Subsp. *pauca*

Subsp. *multiplex*

Subsp. *fastidiosa*
1. Current situation in the Balearic Islands

- Only ST 80 subsp. *pauca*
- Infects olive/wild olive causing a severe decline and mortality
- It causes almond leaf scorch disease
1. Current situation in the Balearic Islands

Mallorca + Menorca

Subsp. *multiplex*

Subsp. *fastidiosa*
How and when did arrive the two strains of *Xylella fastidiosa* to Mallorca?

*Xylella fastidiosa* strains ST1 (subsp. *fastidiosa*) and ST81 (subsp. *multiplex*) were very likely introduced from California with infected almond buds around 1993.
2. Evidences

- Epidemiology
  (aethiology, disease incidence, transmission, pathology, etc.)

- Dendrochronology
  (qPCR + dating growth rings)

- Phylogeny
  (ML & Bayesian trees)
Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain)

D. Gramaje¹, C. Agustí-Brisach¹, A. Pérez-Sierra¹, E. Moralejo², D. Olmo³, L. Mostert⁴, U. Damm⁵, J. Armengol¹

Neofusicoccum parvum  
Pleurosomophora richardsia  
Diplodia olivarum  
Botryosphaeria dothidea  
Diplodia serriata  
Phomopsis amygdali  
Neofusicoccum australe  
Eutypa lata  
Phaeoacremonium iranium  
Collophora hispanica sp.nov  
Phaeoacremonium amigdalinum sp. nov

Phytopathologia Mediterranea (2013) 52, 3, 517–527

RESEARCH PAPERS

Pleurostomophora richardsiae, Neofusicoccum parvum and Phaeoacremonium oleophilum associated with a decline of olives in southern Italy

Antonia CARLUCCIP, Maria Luisa RAIMONDO*, Francesca CIBELLP, Alan J.L. PHILLIPSP and Francisco LOPSP

*Corresponding author.
The link between almond decline caused by fungal trunk pathogens and the ALSD
Currently almond decline is associated with *Xylella fastidiosa*

The link between scorch symptoms and *Xf* infection was straightforward

> 184 positives
The current ALSD incidence and mortality preclude a recent introduction of fungal trunk pathogens.
Understanding the ALSD epidemic

Transmission experiments (n=8) → qPCR+

Grafting experiments (n=13)

Inoculation experiments (n=160) → isolation
➢ Strong association between ALSD and almond dieback and mortality in orchards

➢ Sequence of symptom development correlated (Friedman ANOVA by ranks; $X=3.4$, $P < 0.01$)

➢ ALSD symptoms preceded shoot and branch dieback in 96% of the times
Process of taking wood samples
$dy/dt = yr(1 - y)$

Disease incidence = 0.01
The Kaplan-Meier median (50%) survival estimate was 13 years (25-75% percentil: 12-14 yr)

Table year of first detection

<table>
<thead>
<tr>
<th>Sample</th>
<th>Year</th>
<th>Censor</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOCO 0</td>
<td>2006</td>
<td>complete</td>
</tr>
<tr>
<td>XYL 95</td>
<td>2016</td>
<td>censored</td>
</tr>
<tr>
<td>XYL160</td>
<td>2012</td>
<td>censored</td>
</tr>
<tr>
<td>XYL 192</td>
<td>2008</td>
<td>censored</td>
</tr>
<tr>
<td>XYL 739</td>
<td>1998</td>
<td>censored</td>
</tr>
<tr>
<td>XYL 1602/17 2</td>
<td>&lt;2002</td>
<td>censored</td>
</tr>
</tbody>
</table>

(n = 34 almond trees)

*Xylella fastidiosa subsp. fastidiosa*

*Xylella fastidiosa subsp. multiplex*

No Xylella fastidiosa detected before 1995
Bayesian Phylogenetic analysis

- 44 SNPs
- 14 SNPs

Posterior probability higher than 0.95
Blue: almond genomes
Red: grape genomes
Thanks for your attention!

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1. A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease. Microbial nucleic acids should be found preferentially in those organs or gross anatomic sites known to be diseased, and not in those organs that lack pathology.

2. Fewer, or no, copy numbers of pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease.

3. With resolution of disease, the copy number of pathogen-associated nucleic acid sequences should decrease or become undetectable. With clinical relapse, the opposite should occur.

4. When sequence detection predates disease, or sequence copy number correlates with severity of disease or pathology, the sequence-disease association is more likely to be a causal relationship.

5. The nature of the microorganism inferred from the available sequence should be consistent with the known biological context. Sequence correlates should be sought at the cellular level: efforts should be made to demonstrate specific in situ hybridization of microbial sequence to areas of tissue pathology and to visible microorganisms or to areas where microorganisms are presumed to be located.