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

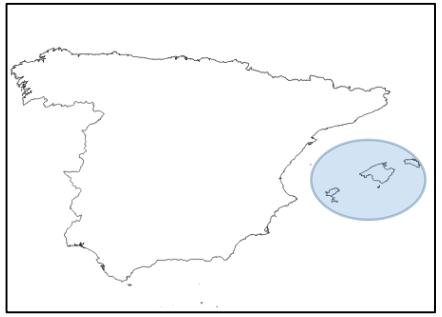
Moralejo E[1], Olmo D[2], M Gomila[3], Nieto A[2], Montesinos M[1], Borràs D[2], Andreu J[2], Landa B B[4]

[1]Tragsa, Empresa de Transformación Agraria, Delegación de Baleares, 07005 Palma de Mallorca, Spain.
[2] Serveis de Millora Agrària, Govern Balear, 07009, Palma de Mallorca, Spain.
[3] Microbiology (Biology Department), University of the Balearic Islands, 07122 Palma de Mallorca, Spain.
[4] Instituto 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



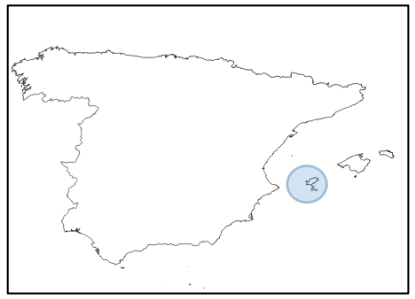
Subsp. *pauca*

Subsp. *multiplex*

Subsp. *fastidiosa*

1. Current situation in the Balearic Islands

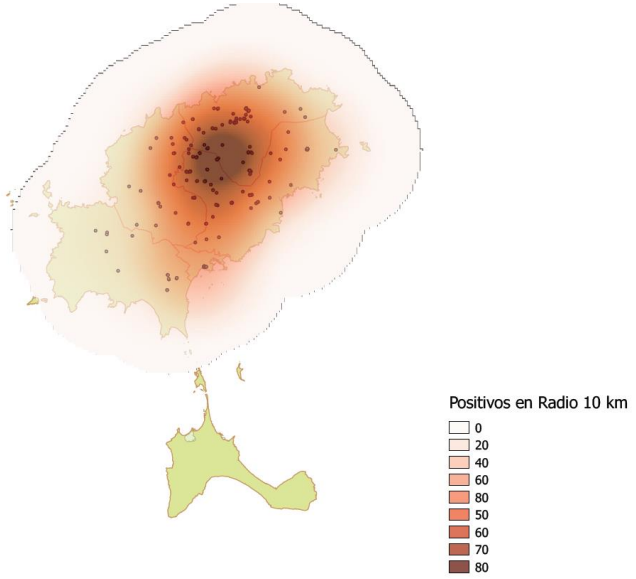
IBIZA



- Only ST 80 subsp. *pauca*
- Infects olive/wild olive causing a severe decline and mortality

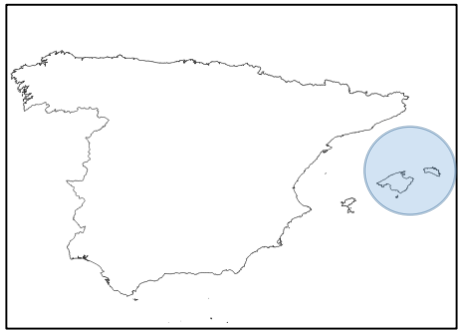
  It causes almond leaf scorch disease

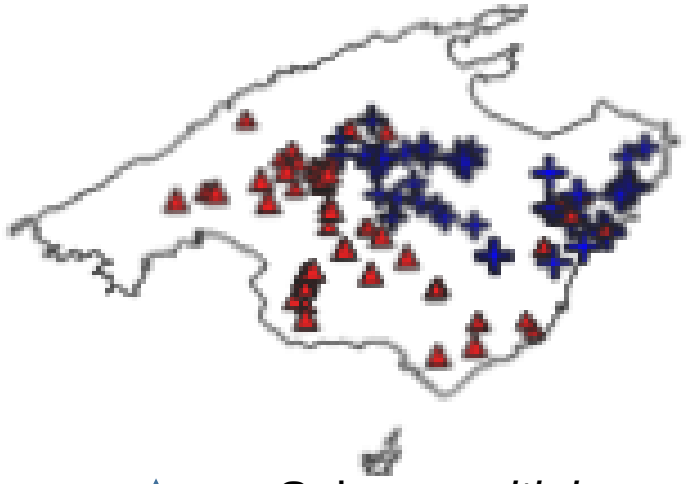
subsp. *pauca*



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 fatidiosa 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)

Present  → Past 

Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain)

D. Gramaje[1], C. Agustí-Brisach[1], A. Pérez-Sierra[1], E. Moralejo[2], D. Olmo[3], L. Mostert[4], U. Damm[5], J. Armengol[1]

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

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

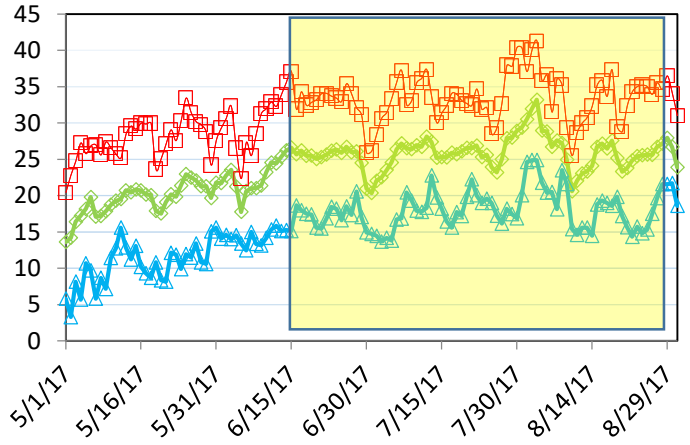
RESEARCH PAPERS

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

Antonia CARLUCCI[1], Maria Luisa RAIMONDO[1], Francesca CIBELLI[1], Alan J.L. PHILLIPS[2] and Francesco LOPS[1]

The link between almond decline caused by fungal trunk pathogens and the ALSD

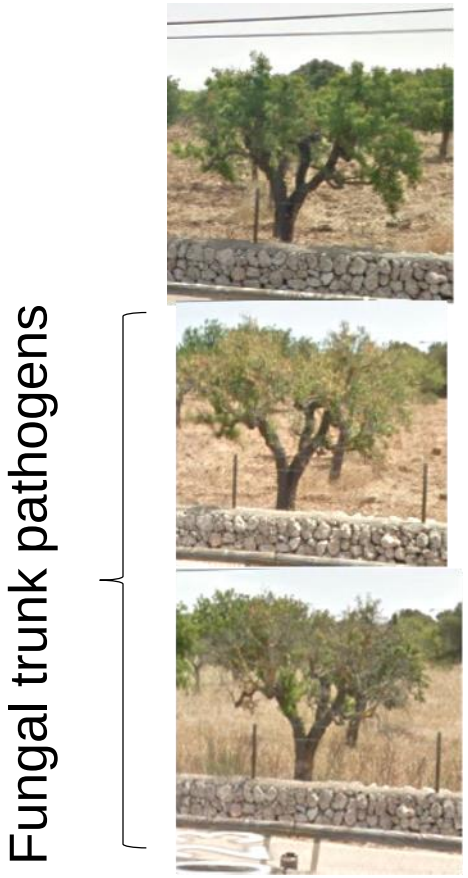
Currently almond decline is associated with *Xylella fastidiosa*

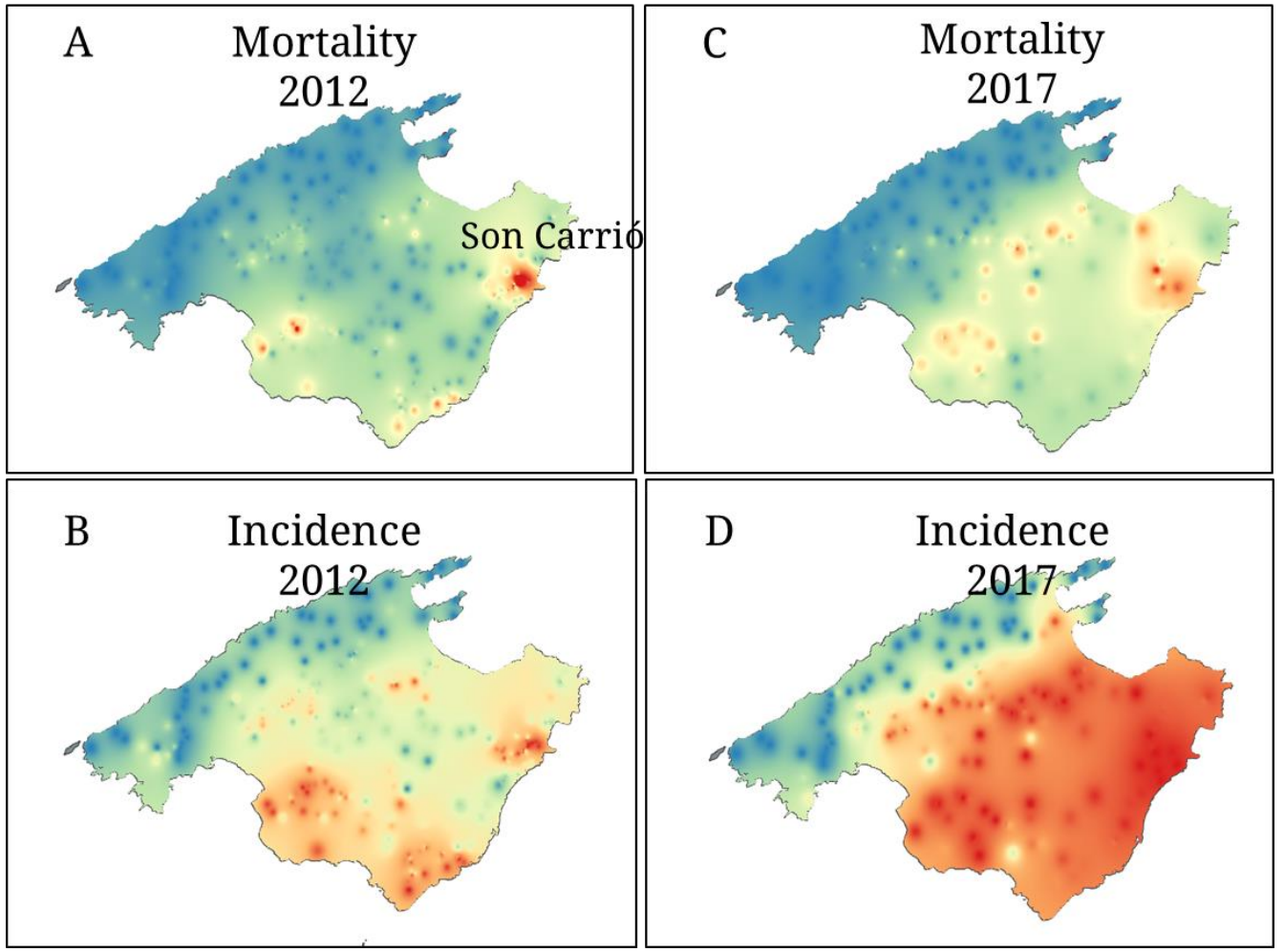


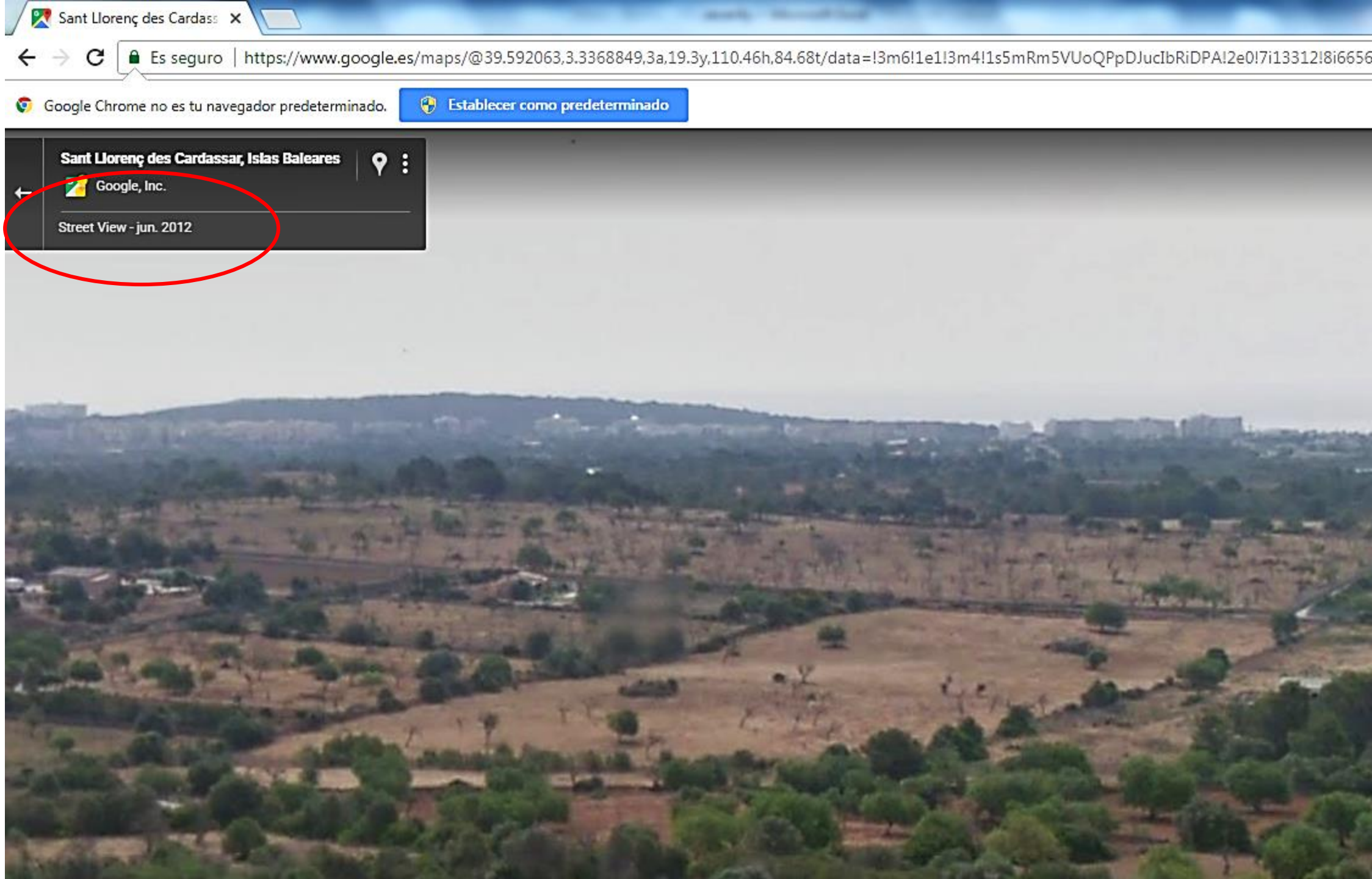
The link between scorch symptoms and *Xf* infection was straightforward

> 184 positives

The current ALSD incidence and mortality preclude a recent introduction






Sant Llorenç des Cardass
Es seguro | https://www.google.es/maps/@39.592063,3.3368849,3a,19.3y,110.46h,84.68t/data=!3m6!1e1!3m4!1s5mRm5VUoQPpDJucIbRiDPA!2e0!7i13312!8i6656
Google Chrome no es tu navegador predeterminado.
Establecer como predeterminado
Sant Llorenç des Cardassar, Islas Baleares
Google, Inc.
Street View - jun. 2012

Understanding the ALSD epidemic

Transmission experiments (n=8)

Grafting experiments (n=13)

Inoculation experiments (n=160)

qPCR+

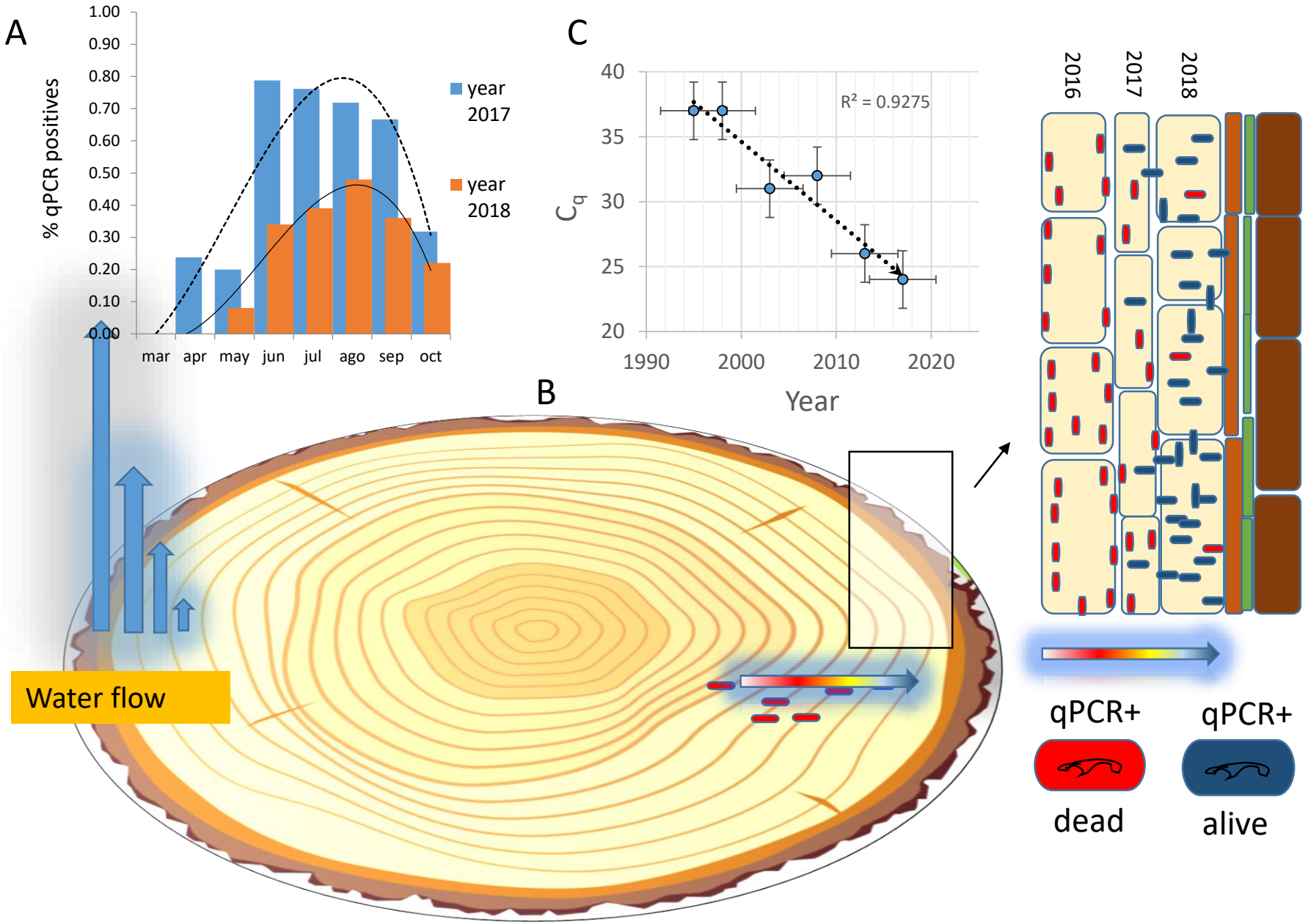
isolation

KOCH'S POSTULTES

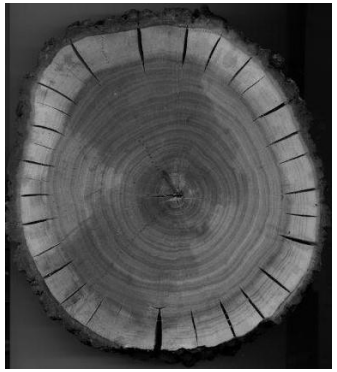
- Strong association between ALSD and almond dieback and mortality in orchards

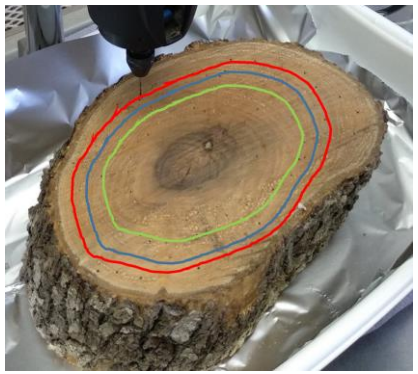
- Sequence of symptom development correlated (Friedman ANOVA by ranks; X=3.4, *P* <0.01)

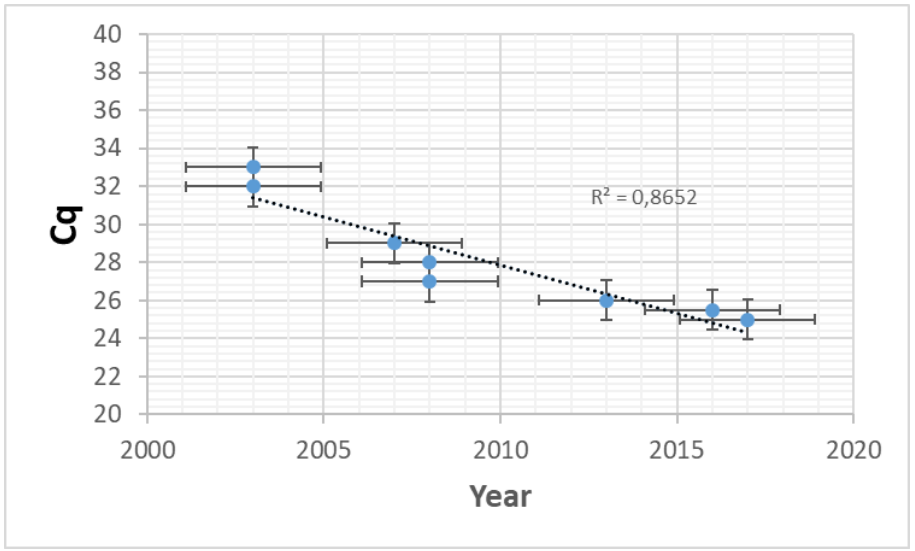
- ALSD symptoms preceded shoot and branch dieback in 96% of the times

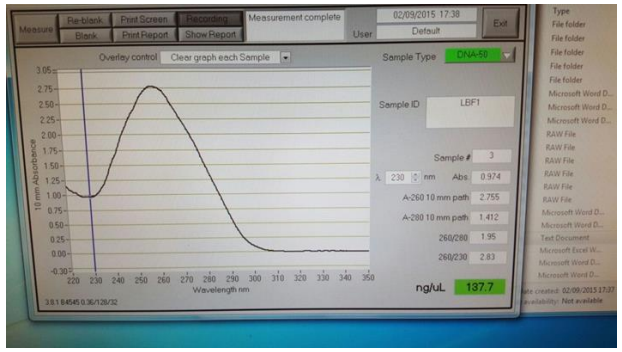

A
% qPCR positives
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
mar
apr
may
jun
jul
ago
sep
oct
year 2017
year 2018
C
C_q
40
35
30
25
20
1990
2000
2010
2020
Year
R^2 = 0.9275
B
Water flow
2016
2017
2018
qPCR+
qPCR+
dead
alive

Process of taking wood samples

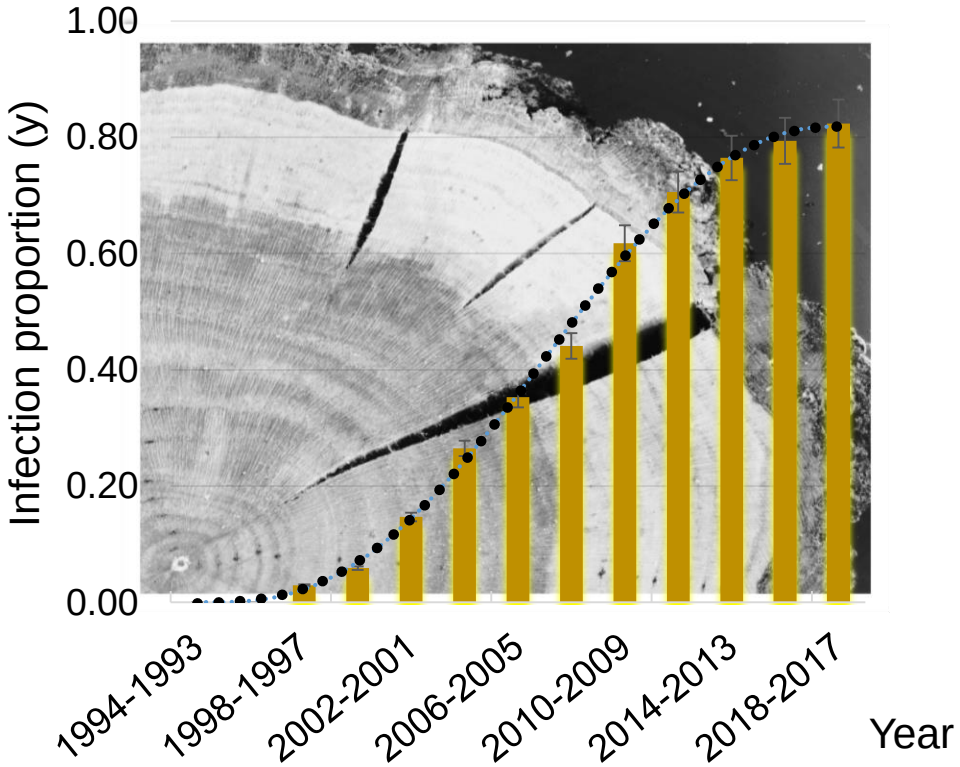


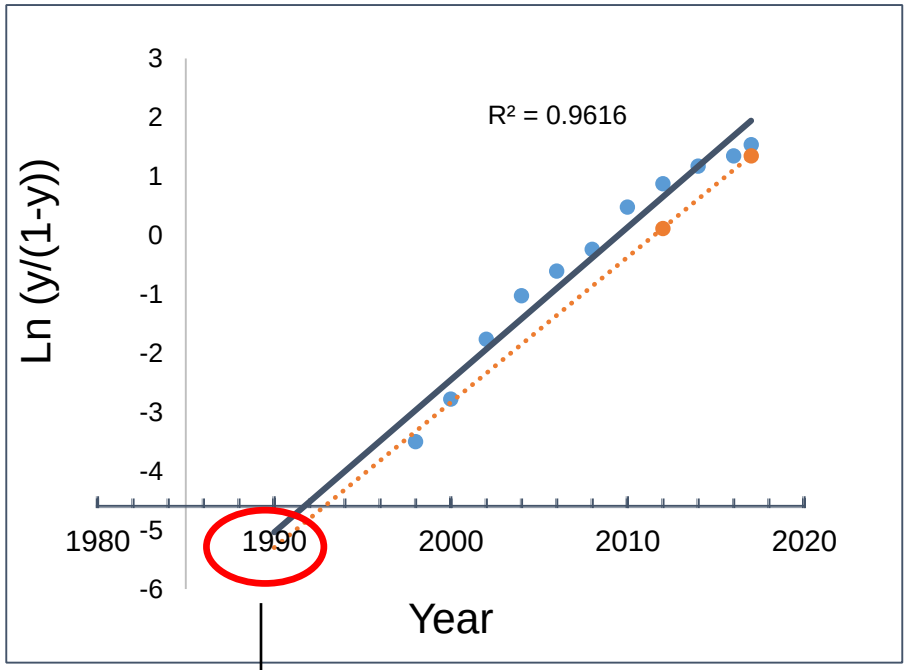






ALSD progress curve





$$dy/dt = yr(1-y)$$

Disease incidence = 0.01

Table year of first detection

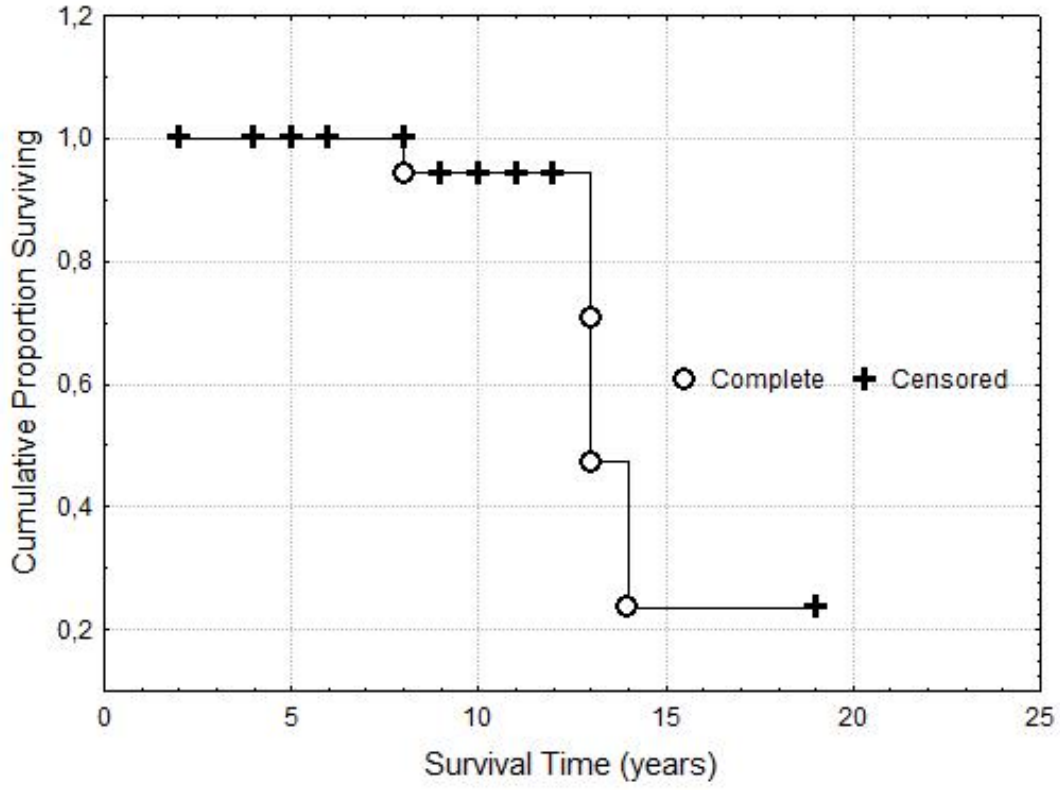
Sample	Year	Censor
FOCO 0	2006	complete
XYL 95	2016	censored
XYL160	2012	censored
XYL 192	2008	censored
XYL 739	1998	censored
XYL 1602/17 2	<2002	censored
....	...	....

(n = 34 almond trees)

Xylella fastidiosa subsp. *fastidiosa*

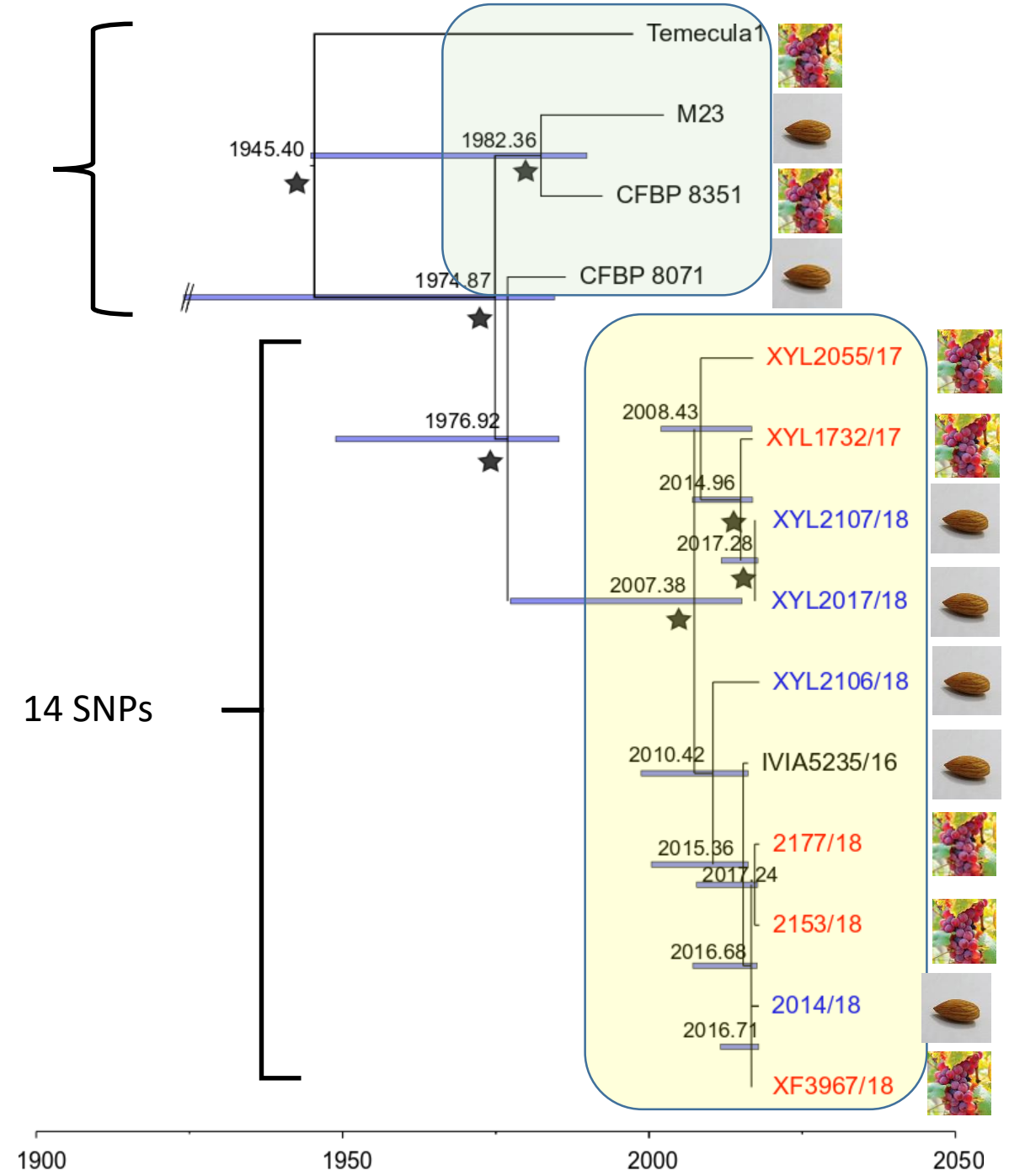
Xylella fastidiosa subsp. *multiplex*

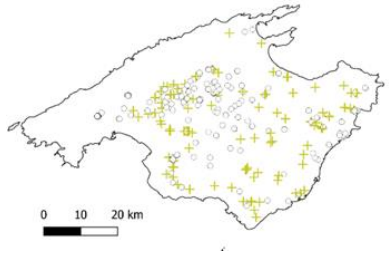
The Kaplan-Meier median (50%) survival estimate was 13 years (25-75% percentil: 12-14 yr)



No *Xylella fastidiosa* detected before 1995

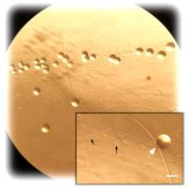
Bayesian Phylogenetic analysis

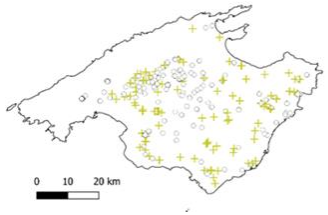


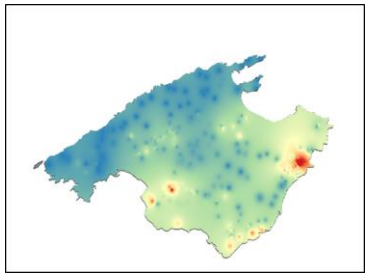


★Posterior probability higher than 0,95
Blue: almond genomes
Red: grape genomes

ST1 & ST81



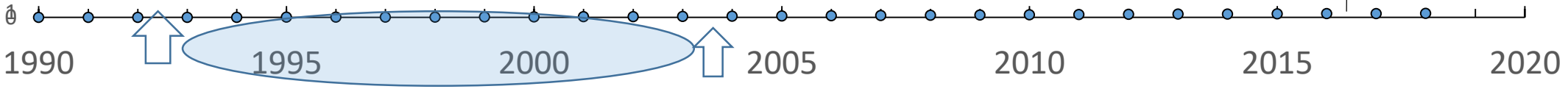




Son Carrió

Erradication ?

X. fastidiosa detection



Thanks for your attention!

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

Moralejo E[1], Olmo D[2], M Gomila[3], Nieto A[2], Montesinos M[1],

Borràs D[2], Andreu J[2], Landa B B[4]

Fredericks DN, & Relman DA (1996). Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. Clinical microbiology reviews, 9 (1), 18-33 PMID: 8665474

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 cTissue-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.