Artificial inoculation of *Xylella fastidiosa* subsp. *pauca* strains in olive plants; an overview of greenhouse experiments.

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INTRODUCTION

Xylella fastidiosa: **Multi host bacteria but causing severe problems in smaller number of plant-based food**
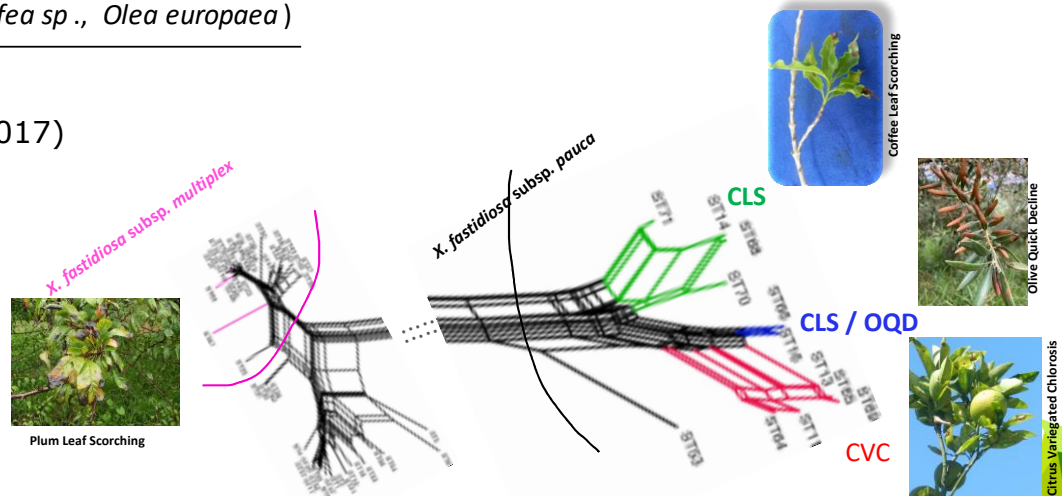
X.f. subsp.	Total Host species	Major Host species*
fastidiosa	4	**4** (*Coffea* sp, *Vitis vinifera* , *Prunus sp* .)
multiplex	51	**4** (*Prunus sp* ., *Olea europaea*)
pauca	28	**3** (*Citrus sinensis, Coffea sp* ., *Olea europaea*)

*Plant-based foods
Adapted from:Baldi & La Porta (Frontier Plant Sci, 2017)

In Brazil:

severe problems with the subsp. *multiplex* and *pauca* causing problems in plant-based food such as coffee, sweet orange, plum and **olive plants**

Coffee Leaf Scorching

Olive Quick Decline

Citrus Variegated Chlorosis

Plum Leaf Scorching

X. fastidiosa subsp. multiplex

X. fastidiosa subsp. pauca

CLS

CLS / OQD

CVC

Coletta-Filho et al., 2017 – Phytopathology

INTRODUCTION

In Brazil: hotspot of diversity of *pauca* strains

Different STs

25*, 71	● *plum (Prunus domestica)*
14, 16, 66, 68	● *coffee (Coffea spp.)*
70	● *hibiscus (Hibiscus rosa-sinensis)*
16**, 84, 85, 86	● *olive (Olea spp.)*
11, 12, 13, 64, 65	● *citrus (Citrus sinensis)*

*ST25 − *X. f.* susbp. *multiplex*

**ST16 − 75% of prevalence
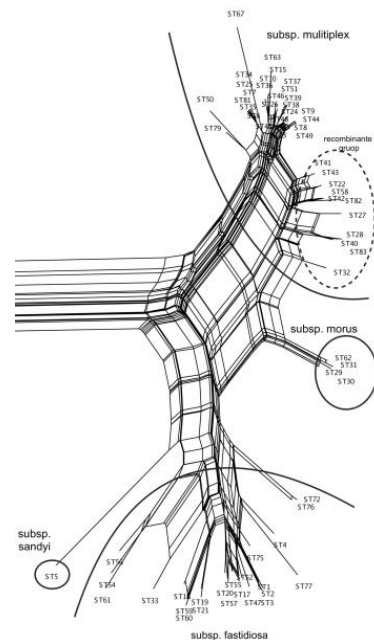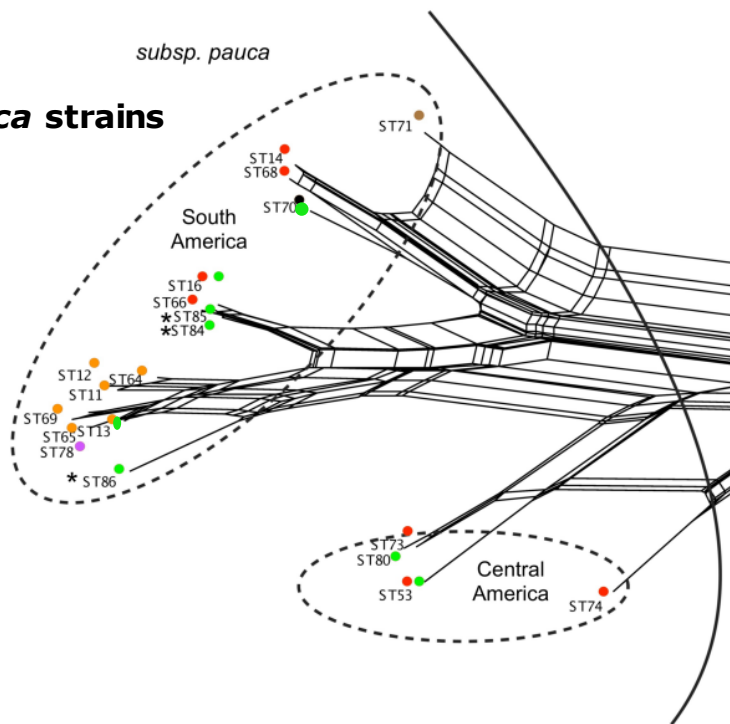
Adapted from Safady et al., 2019 − Phytopathology

Addressed question:

1- Does the olive plant can be infected by others STs from the subsp. *pauca*?

3

METHODOLOGY

1. Rescue the bacteria from -80°C

2. Grown the bacteria on PW or BCYE mediums for 6 – 7 days

3. Resuspended in PBS buffer at 10^7 UFC/ml (OD 1.2 – 1.4)

4. Prinking inoculation in younger branches

  3x weekly ≅ 10 µL / x

  ✔ **Different STs**

  ✔ **Grapollo and Manzalina varieties**

  ✔ **Different plant sizes**

5. *X. fastidiosa* detection by RST31/33 primers
Minsavage et al., (1993)

RESULTS

Summary of 5 different experiments conduced in a period of 30 months

Artificial inoculation in olive trees in green-house by using different STs of *Xylella fastidiosa*

Sequence Type (ST)	Host Origen	Positive infection by artificial inoculation in greenhouse	
		pos/inoc plants	%
X. fastidiosa subsp. pauca			
ST16	*Olea europea*	9/16	56.2
ST84	*Olea europea*	2/5	40.0
ST86	*Olea europea*	1/6	16.6
ST16	*Coffea arabica*	11/17	64.7
ST66	*Coffea arabica*	2/3	66.6
ST11	*Citrus sinensis*	8/16	50.0
ST65	*Citrus sinensis*	6/16	37.5
ST13	*Citrus sinensis*	4/19	21.0
ST70	*Hibiscus rosa-sinensis*	6/15	40.0
ST71	*Prunus domestica*	0/5	00.0
X. fastidiosa subsp. multiplex			
ST25	*Prunus domestica*	0/3	00.0

[1]Number of positive PCR / total of inoculated plants at **60 dai**

Highlights

✔ Most of STs from *pauca* were able to infect olive plants.

✔ None STs of *X. fastidiosa* isolated from Prunus tress (subsp. *multiplex* or *pauca*) was able to infect olive plants.

✔ Variable efficiency of inoculation.

✔ but unfortunately, there were no systemic establishment of bacteria in the host and consequently no symptoms were observed.
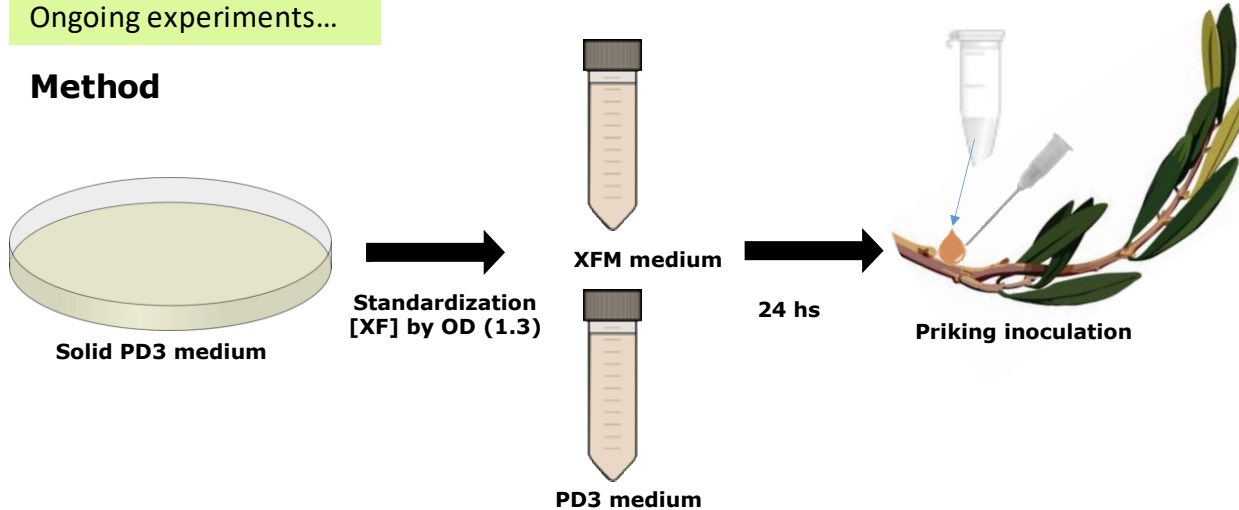
Addressed question

2- Does the passage of *X. fastidiosa* in XFM minimal medium could provide better conditions for the host colonization?

Ongoing experiments…

Method

Solid PD3 medium

Standardization [XF] by OD (1.3)

XFM medium

PD3 medium

24 hs

Priking inoculation

✔ Different STs

✔ Olive, citrus, and tobacco plants

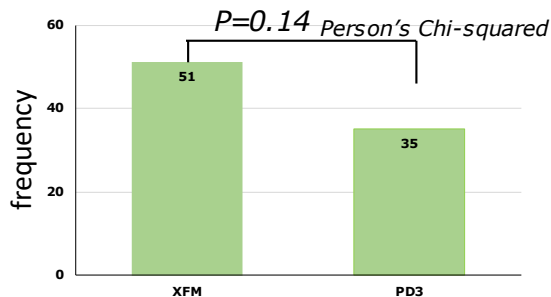✔ *X. fastidiosa* detection by RST31/33 primers

RESULTS

Artificial inoculation ==in olive plants== using *Xylella fastidiosa* strains with passage in minimal - XFM and rich – PD3 mediums

Sequence Type (ST)	Host Origen	Positive infection by artificial inoculation using cells with passage in	
		PD3	XFM
Olive as tested plants			
ST16	*Olea europea*	2/4	1/4
ST84	*Olea europea*	1/5	2/5
ST86	*Olea europea*	1/5	2/5
ST11	*Citrus sinensis*	2/4	3/4
ST13	*Citrus sinensis*	6/14	9/15
ST65	*Citrus sinensis*	0/3	4/5
ST16	*Coffea arabica*	1/4	1/4
ST70	*Hibiscus rosa-sinensis*	2/3	2/5

[1]Number of positive PCR / total of inoculated plants at **180 dai**

Overall frequency of *X. fastidiosa* infection in olive plants

P=0.14 *Person's Chi-squared*

✔ No symptoms in positive PCR olive tree yet, after 240 days from inoculation.

RESULTS

Overall frequency of *X. fastidiosa* infection <mark>in citrus plants</mark> using passage of bacteria in minimal - XFM and rich – PD3 mediums

P=0.08 *Person's Chi-squared*
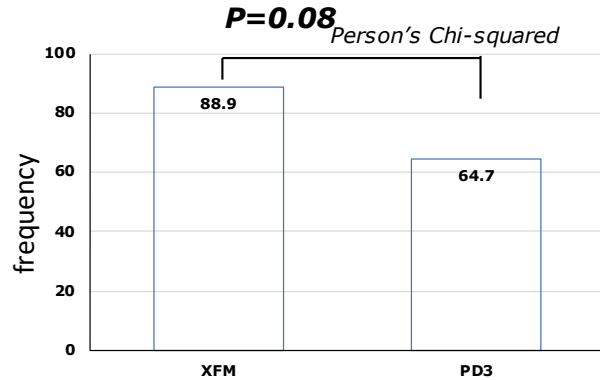
Analysis at **180 dai**

✔ **5 STs:** 11, 13-2x – from CVC
84 and 85 – from OQDS

✔ **XFM:** 20 inoculated plants
PD3: 17 inoculated plants

<mark>CVC symptoms</mark> in *X. fastidiosa*-PCR positive at **240 dai**

Medium	Number of plants with CVC symptoms	Severity		
		Low	Medium	High
XFM	14	6	6	2
PD3	4	1	2	1

P=0.001 *Person's Chi-squared*

Low   Medium   High

RESULTS

Artificial inoculation in tobacco plants using *Xylella fastidiosa* strains with passage in minimal - XFM and rich – PD3 mediums

Sequence Type (ST)	Host Origen	Positive infection by artificial inoculation using cells with passage in	
		PD3	XFM
Tobaco as tested plants			
ST13	*Citrus sinensis*	3/3	3/3
ST84	*Olea europea*	2/3	2/3

[1]Number of positive PCR / total of inoculated plants at **90 dai**

Symptoms in tobacco

Reduction of plant tall

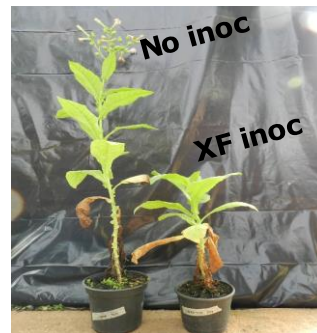| Sequence Type inoculated	Plant height (cm)	
	PD3	XFM
ST13	28 (1.53)[1]	29 (4.36)
ST 84	38 (2.52)	45 (3.00)
Non inoculate	86 (4.58)	

[1]Standard deviation

On leaves

Brown spots on the leaves

ST13

ST84

RESULTS

✔ Passage of *X. fastidiosa* in minimal medium - XFM improves the infection efficiency mainly for the resilient plants for infection in greenhouse such as olive and citrus.

✔ The CVC symptom was significantly increase by the passage of bacteria in XFM medium.

✔ For our conditions, the latency of OQDS in greenhouse conditions seems to be longer mainly when *Xylella fastidiosa* is artificially inoculated.

By rooting XF-infected but asymptomatic sprouts -> **3.5 years in greenhouse to show the OQD symptoms**.

Thanks for your attention

Acknowledgments: