Xylella fastidosa in olives trees in Brazil: survey, host susceptibility, and artificial inoculation testes

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efsa European Food Safety Authority
UIB Universitat de les Illes Balears
European Commission
PONTE Pest Organisms Threatening Europe
XF ACTORS
Euphresco

Insertion in XF-ACTORS project

WP 2- Biology and genetics of relevant *X. fastidiosa* strains

Work package number	2		Lead beneficiary P4 INRA – Marie-Agnes Jacques						
Work package title	Biology and genetics of relevant *X. fastidiosa* strains								
Participant number	P1	P3	P4	P5	P9	P10	P11	P12	P13
Short name of participant	CNR	UNIBA	INRA	CSIC	ILVO	UC	IAC	NTU	UCR
Person/months per participant:	15	8	43	8	40	18	17	24	24
Start month: 1					End month: 42				

Task 2.1 Host range and pathogenicity of *Xf* strains relevant for EU (P1, P4, P5, P9, P11)

Task 2.2 Population genetics of *Xf* strains relevant for EU (P1, P5, P10, P11, P12, P13)

The Brazilian olive oil industry

✔ **Young tress (<15 y old)**

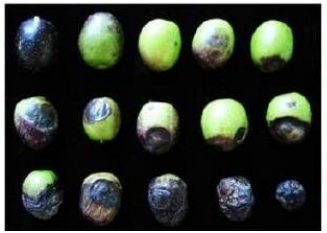
✔ **Low experience with crop**

✔ **Cultivated at high altitudes**

>700 m at sea level

🡻Temp. for flowering

🡹Humidity



Fungi diseases

✔ **Unknown Disorders**

Biotic and Abiotic

Diseases: OQD - *Xylella fastidiosa*

Phytopathologia Mediterranea (2016) temp3–8
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NEW OR UNUSUAL DISEASE REPORTS

First report of olive leaf scorch in Brazil, associated with *Xylella fastidiosa* subsp. *pauca*

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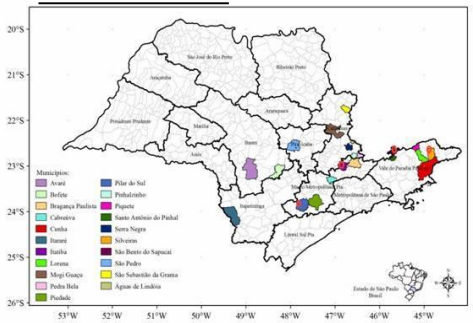
Xylella fastidiosa in olive trees in Brazil: Survey, host susceptibility and artificial inoculation tests

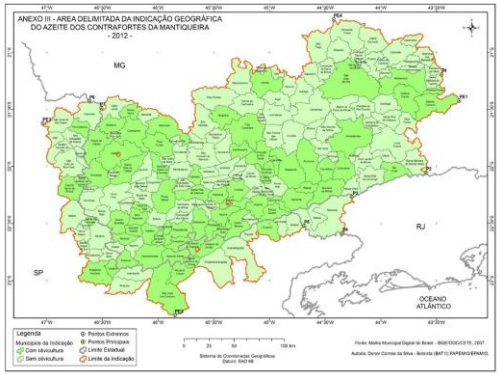
Survey: Spatial distribution of XF and OQD

Olive orchards areas

Sao Paulo St

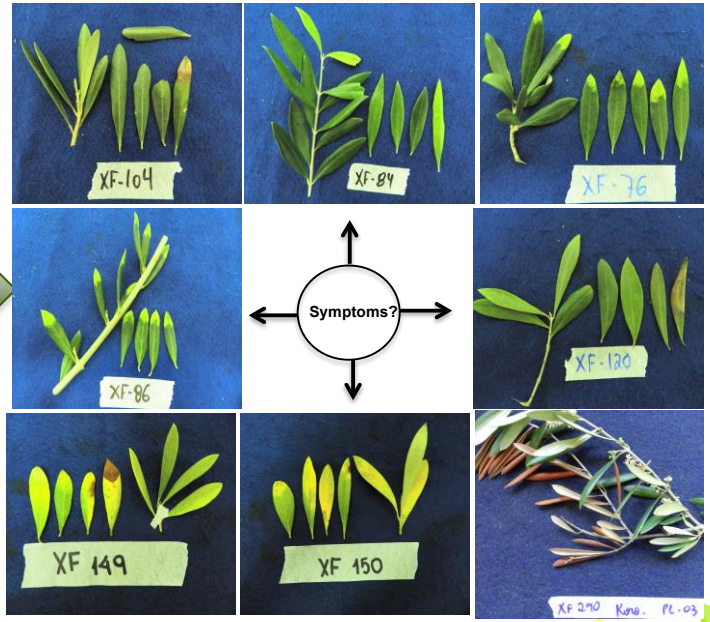


Minas Gerais St



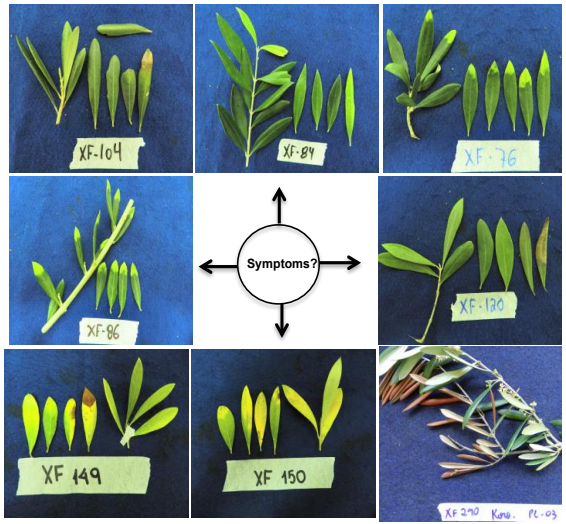
Samples collected

Register by photos



Xylella fastidiosa in olive trees in Brazil: Survey, host susceptibility and artificial inoculation tests

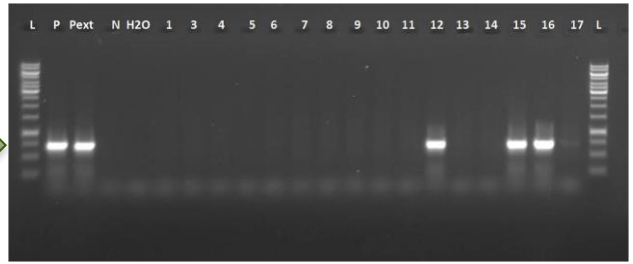
Survey: Spatial distribution of XF and OQD



DNA extraction

PCR – RST31/RST33 primers

Isolation on BCYE medium



✔ **Low experience with crop**

✔ **Cultivated at high altitudes**

>700 m at sea level

↓Temp. for flowering

↑Humidity

✔ **Unknown Disorders**

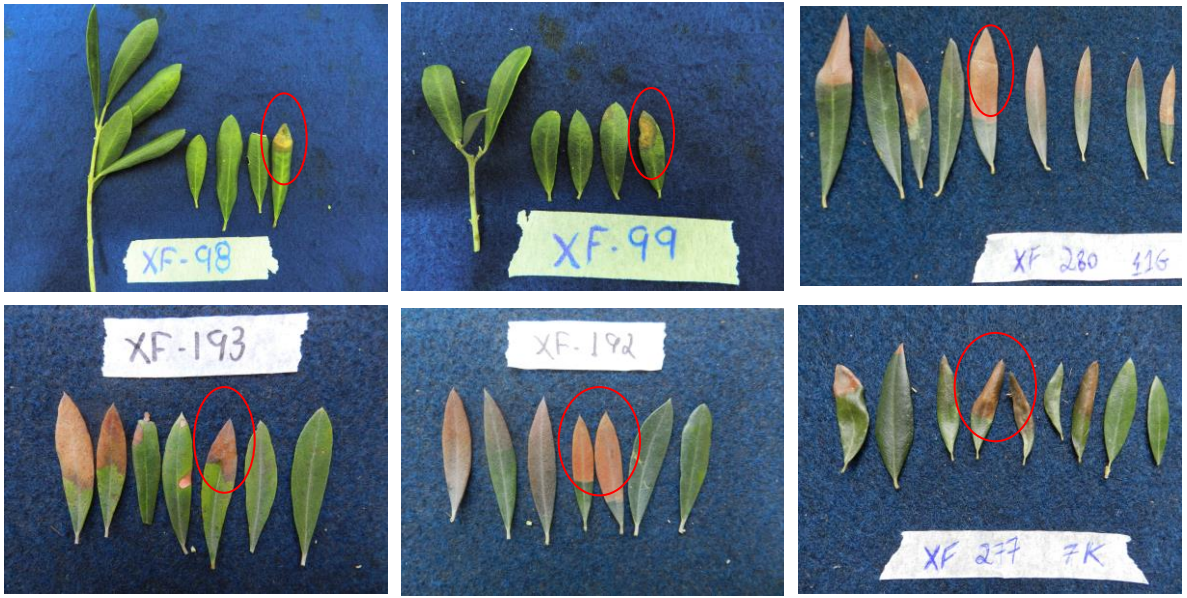
Biotic and Abiotic

✓Survey

✓ What specific symptoms are associated?

Survey: Spatial distribution of XF and OQD

✓ What specific symptoms are associated?



82% PCR +

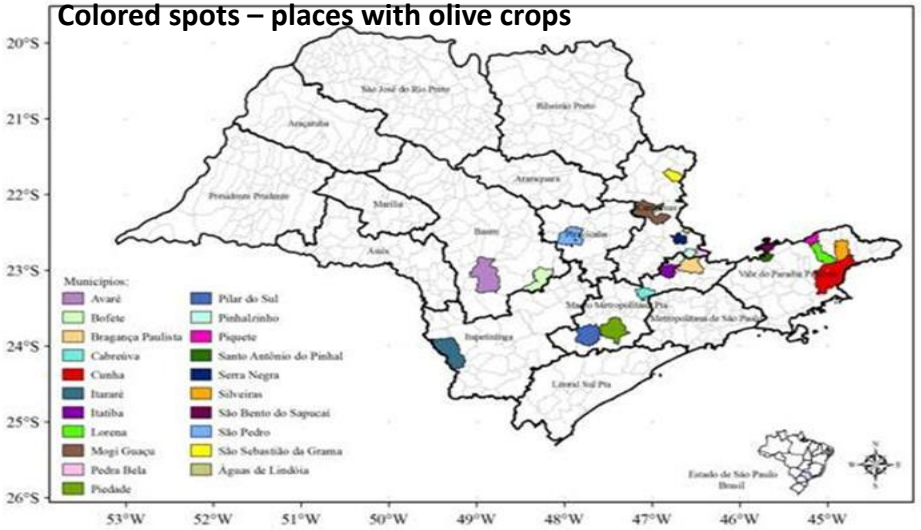
No *Xf* positive

Xylella fastidiosa in olive trees in Brazil: Survey, host susceptibility and artificial inoculation tests

Survey: Spatial distribution of XF and OQD

Origin (Altitude)	Sampling Date	Total / Positive
Sao Paulo State		
Area 1 (1512m)	Apr-16	05 (05)
	Apr-17	07 (02)
	Aug-17	10 (05)
		54%
Area 2 (700m)	Nov-15	08 (03)
	Dec-15	08 (06)
	Sep-15	09 (03)
		48%
Area 3 (883m)	May-17	10 (02)
	Sep-17	08 (01)
		17%
Area 4 (750m)	Apr-17	19 (03)
		16%
Area 5 (615m)	Dec-15	05 (05)
		100%

Olive orchards areas



≅400 hectares – Sao Paulo State

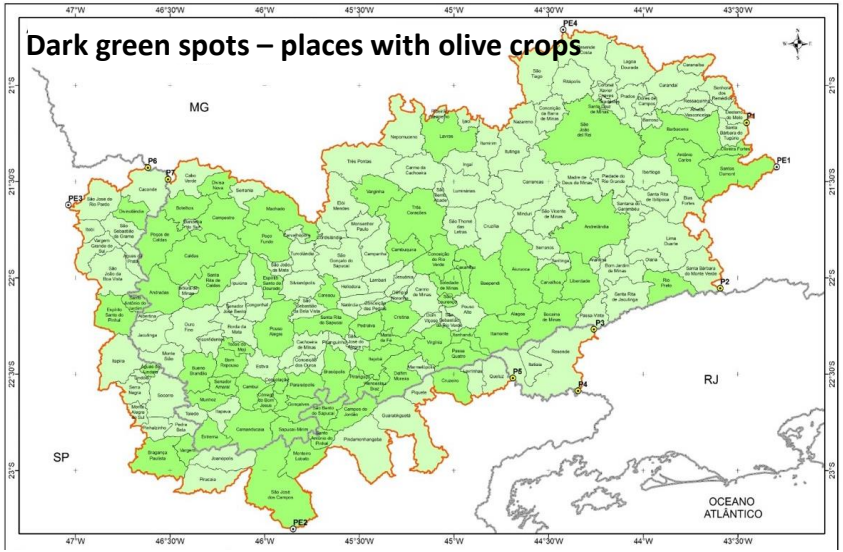
- **XF and disease plants were found in all sampled orchards**
- **Not all plants with regular scorched leaves were PCR positive for XF**

Xylella fastidiosa in olive trees in Brazil: Survey, host susceptibility and artificial inoculation tests

Survey: Spatial distribution of XF and OQD

Origin (Altitude)	Sampling Date	Total / Positive
Minas Gerais State		
Area 1 (1320m)	Nov-15	11 (01)
	Jan-17	74 (64)
	Feb-17	19 (11)
	Apr-17	10 (07)
	Apr-17	12 (05)
	Apr-17	06 (00)
		74%
Area 2 (1170m)	Sep-16	04 (04)
	Oct-17	08 (02)
		50%
Area 3 (1166m)	Apr-17	02 (00)
	Aug-17	04 (00)
	Oct-17	11 (05)
		29%
Area 4 (1440m)	Oct-17	12 (02)
		12%

Olive orchards areas



≅600 hectares –MG State

- **XF and disease plants were found in all sampled orchards**

- **Not all plants with regular scorched leaves were PCR positive for XF**

Xylella fastidiosa in olive trees in Brazil: Survey, host susceptibility and artificial inoculation tests

Host susceptibility: different olives varieties

Survey of *Xf* in Germoplasm collection naturally infected by *Xf*

✔ 43 off 50 (86%) olive varieties were PCR positive (Pos) for *Xf.*

✔ The majority of PCR-Pos plants were asymptomatic (no leaves scorching), yet.

✔ *Xf* was isolate from 21 off 43 PCR-Pos plants.

Germoplasm collection

Genotype	PCR	Culturing	Genotype	PCR	Culturing
Arbequina	Pos	Pos	JB-1	Pos	Pos
Alto D'ouro	Pos	Pos	JB-1 A2	Pos	---
Alto D'ouro A2	Pos	---	Koronake	Pos	Pos
Arbequina	Pos	Pos	Leccino	Pos	---
Arbosana	Pos	---	Lechin de Sevilha	Pos	Pos
Ascolano 315	Pos	Pos	Manzanilla 215	Pos	Pos
Cerignola	Pos	---	Manzanilla 234	Pos	---
Clone 0025	Pos	---	Manzanilla Denite	Pos	---
Clone 0080	Pos	---	Manzanilla Reina	Pos	---
Clone 113	Pos	---	Manzanilla Reina A1	Pos	---
Coratina	Pos	Pos	Maria da Fé	Pos	Pos
Cormicabra	Pos	Pos	Mission	Pos	Pos
Frantoio	Pos	---	Mission 293	Pos	---
Galega	Pos	---	Mistura	Pos	---
Galega A2	Pos	Pos	Moraiolo	Pos	---
Galega A3	Pos	---	Penafiel SP	Pos	Pos
Grappolo 541	Pos	Pos	Picual	Pos	---
Grappolo 550	Pos	---	Ropades 393	Pos	Pos
Grappolo 561	Pos	---	Ropades 398	Pos	---
Grappolo 575	Pos	---	Saialima Gloub	Pos	---
Grousse Alberkan 39	Pos	---	Santa Catalina	Pos	---
Salome 448	Neg	---	Tafahi 391	Pos	---
Negroa	Neg	---	Empeltre	Neg	---
Zalmate 0012	Neg	---	Gordal de Sevilha	Neg	---
Grappolo 553	Neg	---	Barnea	Neg	---

Xylella fastidiosa in olive trees in Brazil: Survey, host susceptibility and artificial inoculation tests

Host susceptibility: different olives varieties

Next steps

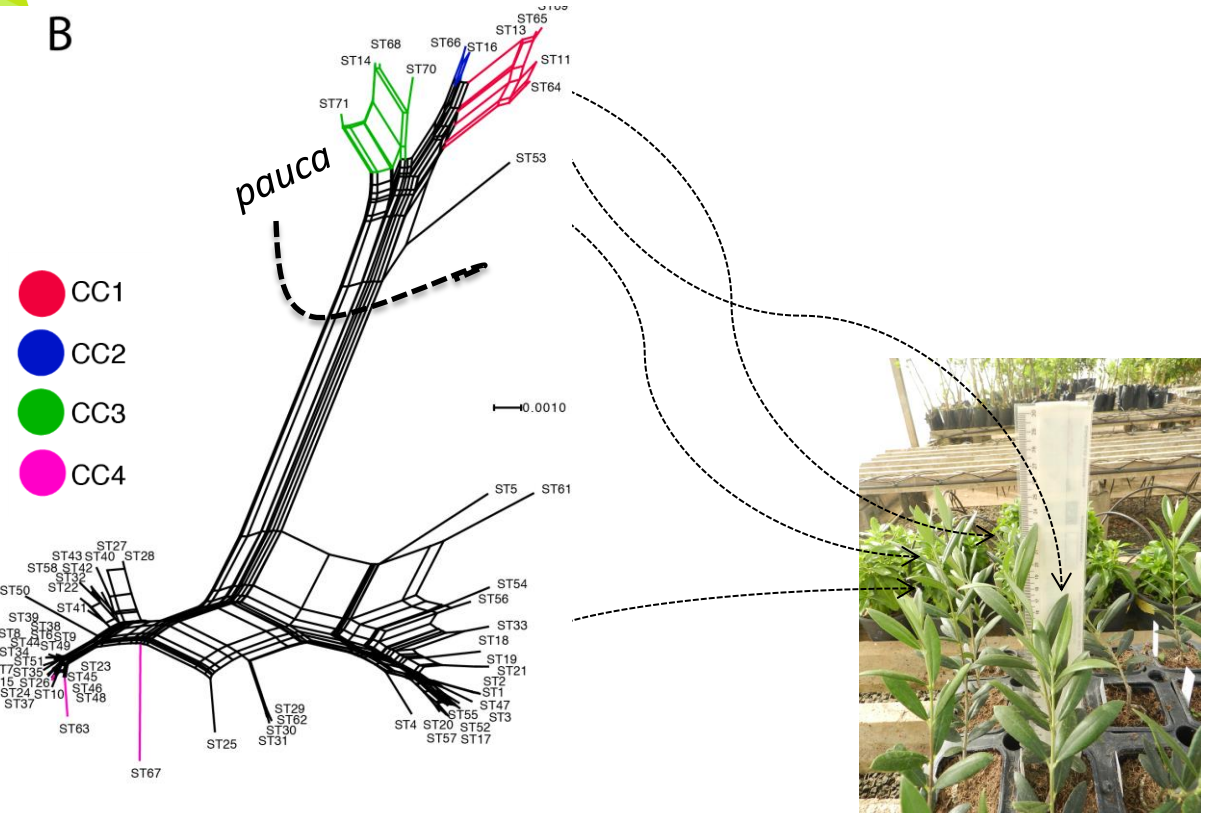
✔ PCR-Neg plants have been checked for *Xf*

✔ PCR-Pos but asymptomatic plants have been checked for specific leaves scorching associated symptoms.

Germoplasm collection

Genotype	PCR	Culturing	Genotype	PCR	Culturing
Arbequina	Pos	Pos	JB-1	Pos	Pos
Alto D'ouro	Pos	Pos	JB-1 A2	Pos	---
Alto D'ouro A2	Pos	---	Koronake	Pos	Pos
Arbequina	Pos	Pos	Leccino	Pos	---
Arbosana	Pos	---	Lechin de Sevilha	Pos	Pos
Ascolano 315	Pos	Pos	Manzanilla 215	Pos	Pos
Cerignola	Pos	---	Manzanilla 234	Pos	---
Clone 0025	Pos	---	Manzanilla Denite	Pos	---
Clone 0080	Pos	---	Manzanilla Reina	Pos	---
Clone 113	Pos	---	Manzanilla Reina A	Pos	---
Coratina	Pos	Pos	Maria da Fé	Pos	Pos
Cormicabra	Pos	Pos	Mission	Pos	Pos
Frantoio	Pos	---	Mission 293	Pos	---
Galega	Pos	---	Mistura	Pos	---
Galega A2	Pos	Pos	Moraiolo	Pos	---
Galega A3	Pos	---	Penafiel SP	Pos	Pos
Grappolo 541	Pos	Pos	Picual	Pos	---
Grappolo 550	Pos	---	Ropades 393	Pos	Pos
Grappolo 561	Pos	---	Ropades 398	Pos	---
Grappolo 575	Pos	---	Saialima Gloub	Pos	---
Grousse Alberkan 39	Pos	---	Santa Catalina	Pos	---
Salome 448	Neg	---	Tafahi 391	Pos	---
Negroa	Neg	---	Empeltre	Neg	---
Zalmate 0012	Neg	---	Gordal de Sevilha	Neg	---
Grappolo 553	Neg	---	Barnea	Neg	---

Artificial inoculation tests: Different STs of subsp. *pauca* in olive plants



Needle inoculation

3x weekly ≅ 10 µL / time

Total 30 µL of 10^7 UFC/ml

Artificial inoculation tests: Different STs of subsp. *pauca* in olive plants

Experiment 1

Strain / ST		Host origin	60 DAI	120 DAI	360 DAI
MF01 / ST16 CC2	PCR	*Olea europea*	3/5	2/5	2/5
	Symptoms		0/5	0/5	0/5
9a5c / ST11 CC1	PCR	*Citrus sinensis*	0/5	0/5	0/5
	Symptoms		0/5	0/5	0/5
PR8X / ST71 CC3	PCR	*Prunus domestica*	0/5	1/5	1/5
	Symptoms		0/5	0/5	0/5

CC2: (ST16) - Infection but no symptoms under greenhouse, so far!

CC3: (ST71) ?

Artificial inoculation tests: Different STs of subsp. *pauca* in olive plants

Experiment II

Strain/ST		Host origin	60 DAI	120 DAI	360 DAI
MF01/ST16					
CC2	PCR	*Olea europea*	2/3	2/3	2/3
	Symptoms		0/3	0/3	0/3
COF1.9/ST16					
CC2	PCR	*Coffea arabica*	3/3	2/3	2/3
	Symptoms		0/3	0/3	0/3
COF9/ST66					
CC2	PCR	*Coffea arabica*	2/3	1/3	2/3
	Symptoms		0/3	0/3	0/3

CC2 (STs16 and 66) - Infection but no symptoms under greenhouse, yet!

Artificial inoculation tests: Different STs of subsp. *pauca* in olive plants

Under development experiments

- ✔ Greenhouse with control of temperature
  - ➔ Minimize the temperature range
- ✔ Using hosts with different size (age)

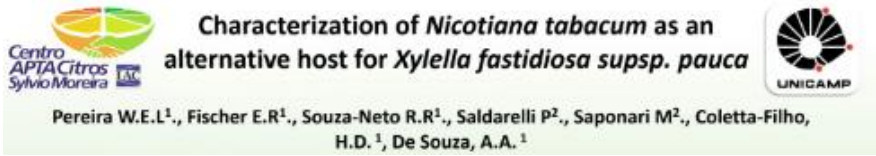
Using different strains in the same plant.

- ➔ each strain has been inoculated in specific branch

Artificial inoculation tests: tobacco response to *X.f.* subsp *pauca* strains

Artificial inoculation tests: tobacco response to *X.f.* subsp *pauca* strains

More details in Poster session…..



Conclusion:

✔ Xf is spread over the majority of olive orchards in Southeast Brazil (Sao Paulo and Minas Gerais States).

In tropical clime like Brazil, olive leaves scorching symptoms must be more detailed to be used as a key of *Xf* infection .

Xf PCR-positive samples ranged from: 16 to 100% (SP state)
12 to 74% (MG state) .

✔ Samples collect in summer time (Dez – Fev) seem to be more infected

Conclusion:

✔ All commercial olive varieties were found infected by *Xf* in field with high inoculum pressure.

✔ Successful artificial inoculations were obtained only with strains (ST) from CC2 of *X. fastidiosa* subsp. *pauca,* so far.

Conclusion:

✔ *Xf* strains (ST) shown variable virulence (ST13 > ST16 > ST53) using tobacco as model plant.

✔ The small dark spot lesions on leaves, like described in literature, was observed only for 9a5c (ST13) tobacco-inoculated plants

✔ The tobacco variety “RP1” (local selection) was more sensitive to inoculation with all *Xf* strains and shown more clear phenotype compared to “Havana”.

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Thanks for your attention

Acknowledgments:

XF ACTORS
727987

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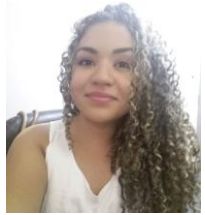
Und grad stud

Emile

Und grad stud

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