

Spatial distribution and genetic structure of *X. fastidiosa* subsp. *pauca* in Olive trees in Southeast Brazil.

Nágela G. Safady¹, Soares KC¹, Armange E¹., Silva LFO², Lopes JRS³, **Coletta-Filho HD¹**

✉ hdcoletta@ccsm.br

¹Centro de Citricultura “Sylvio Moreira”/ Instituto Agronômico de Campinas – IAC/ São Paulo State, Brazil; ²EPAMIG- Maria da Fé/ Minas Gerais State, Brazil; ³Universidade de São Paulo (ESALQ/USP)/ São Paulo State, Brazil

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

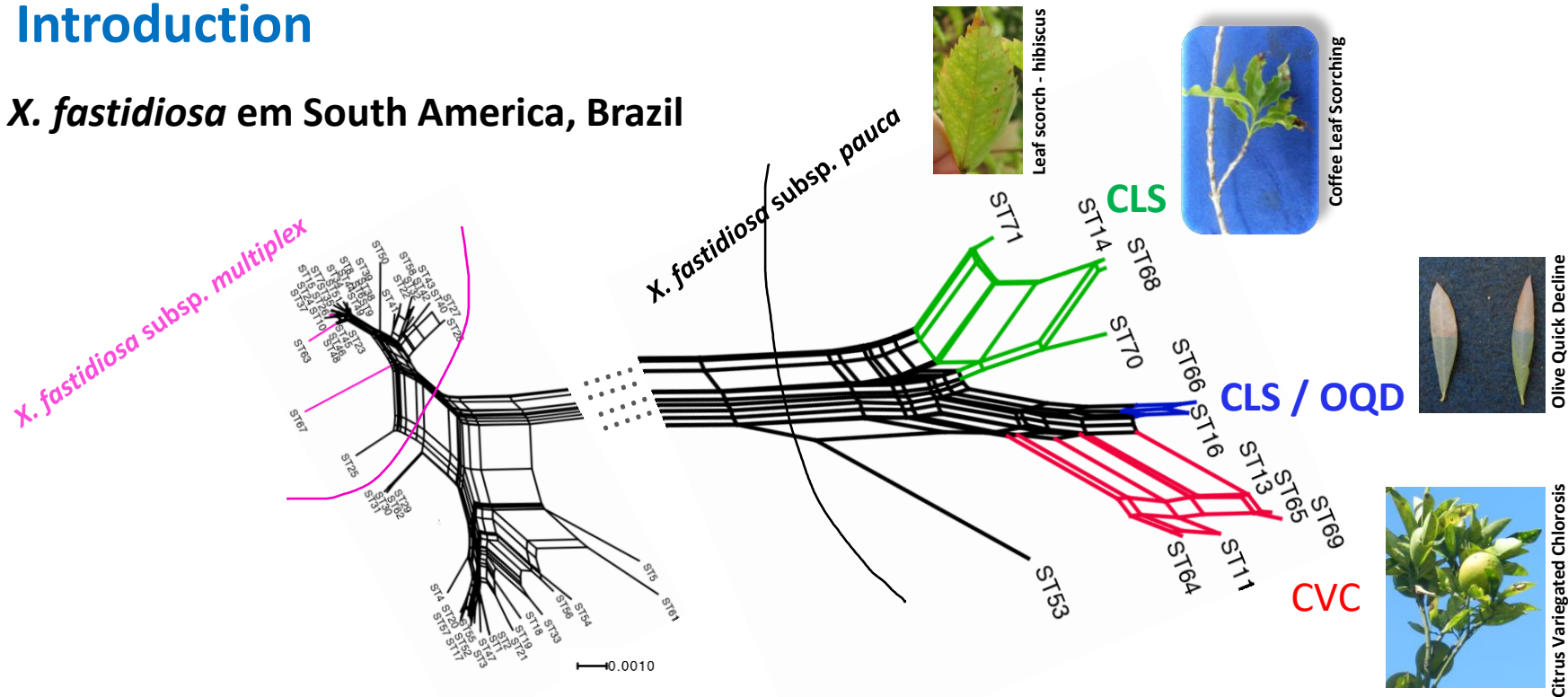
Work package number	2		Lead beneficiary P4 INRA – Marie-Agnes Jacques							
Work package title	Biology and genetics of relevant <i>X. fastidiosa</i> strains									
Participant number	P1	P3	P4	P5	P9	P10	P11	P12	P13	
Short name of participant	CNR	UNIBA	INRA	CSIC	ILVO	UC	IAC	NTU	UCR	
Person/months per 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)

Introduction

X. fastidiosa em South America, Brazil

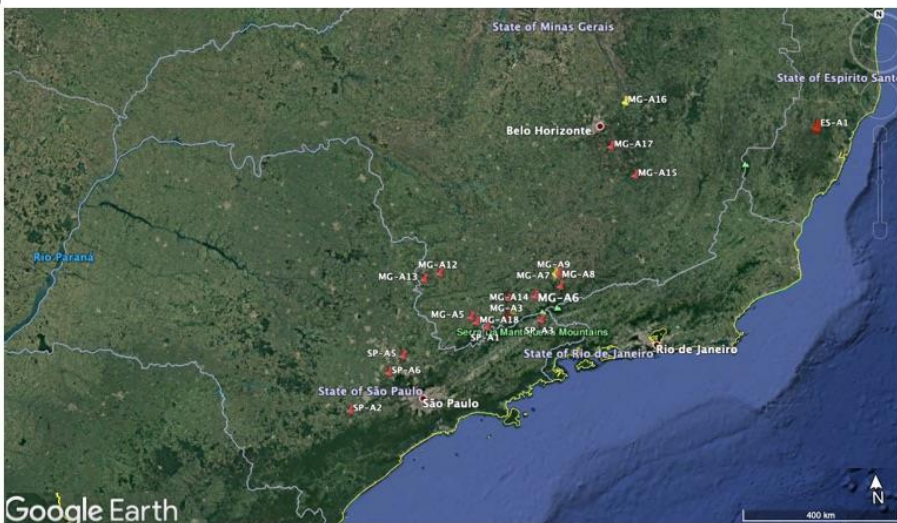


Introduction

X. fastidiosa is spread over geographically distant olive orchards, in Br

Xf in olive was first reported in 2016

85% of surveyed orchards were positive for *X. fastidiosa*.



Yellow pins – negative for XF

Red pins – positive for XF

Adapted from Safady et al., 2019 – Phytopathology



Introduction

MLST haplotypes in field

- ST 16: 75% of prevalence*
- Others ST (84, 85, 86, 13, and 70)*
: rare frequency

*Natural infection in olive trees in field



Artificial inoculation in olive trees in green-house
by using different ST from subsp. *pauca*.

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

Olives orchards are located at different altitudes and environment

Introduction

Addressed question:

What is distribution of genetic diversity in *X. fastidiosa* populations across a hierarchy of geographic scales?

The fast-evolving simple sequence DNA repeat (SSR) markers is the most adequate tools by the well know resolution.

Origin (State/area) ^a	Latitude / Longitude	Altitude (m)	Landscape Vegetation
Minas Gerais-A1	22°18'56.55" / 45°22'30.49"	1300	Araucaria forest
Minas Gerais-A2	22°35'46.45" / 45°55'25.88"	1235	Araucaria and ombrophilous forests
Minas Gerais-A3	22°10' 47.57" / 44°36'17.71"	1200	Araucaria and ombrophilous forests
Minas Gerais-A4	22°59'52.60" / 44°40'27.31"	1400	Araucaria and ombrophilous forests
Minas Gerais-A5	22°01'5.55" / 44°39'32.90"	1500	Araucaria and ombrophilous forests
Minas Gerais-A7	22°01'8.58" / 44°37'23.17"	1300	Araucaria and ombrophilous forests
Minas Gerais-A10	22°36'53.30" / 45° 24'32.87"	1780	Araucaria and ombrophilous forests
Minas Gerais-A13	22°30'29.87" / 44°18'7.09"	1250	Ombrophilous forests
Minas Gerais-A12	21°57'31.16" / 46° 21'19.96"	1380	Ombrophilous forests, grass
Minas Gerais-A13	21°1'54.44" / 46°35'3.77"	1050	Araucaria and ombrophilous forests
Minas Gerais-A14	22°18'16.85" / 44°58' 34.03"	1065	Small area of ombrophilous forests, grasse
Minas Gerais-A15	20°41'7.35" / 43°28' 9.34"	854	Ombrophilous forests
Minas Gerais-A16	19°40'20.63" / 43°35'11.67"	987	Ombrophilous forests
Minas Gerais-A17	20°19'54.88" / 43°45'19.93"	883	Ombrophilous forests, grasses
Minas Gerais-A18	22°35'8.84" / 45°50'33.50"	1329	Araucaria and ombrophilous forests
São Paulo- A1	22°42'10.71" / 45°41'32.91"	1602	Araucaria and ombrophilous forests
São Paulo- A2	23°45'37.30" / 47°44'45.47"	690	Small area of tropical forests
São Paulo- A3	22°37'41.72" / 44°53'55.70"	615	Ombrophilous forests, grasses
São Paulo- A4	22°30'13.54" / 46°36'45.39"	1054	Araucaria forest
São Paulo- A5	23°31'13.64" / 46°56'11.21"	755	No vegetation, grasses
São Paulo- A6	23°16'22.23" / 47°09'9.42"	883	Pine forest, eucalyptus, grasses

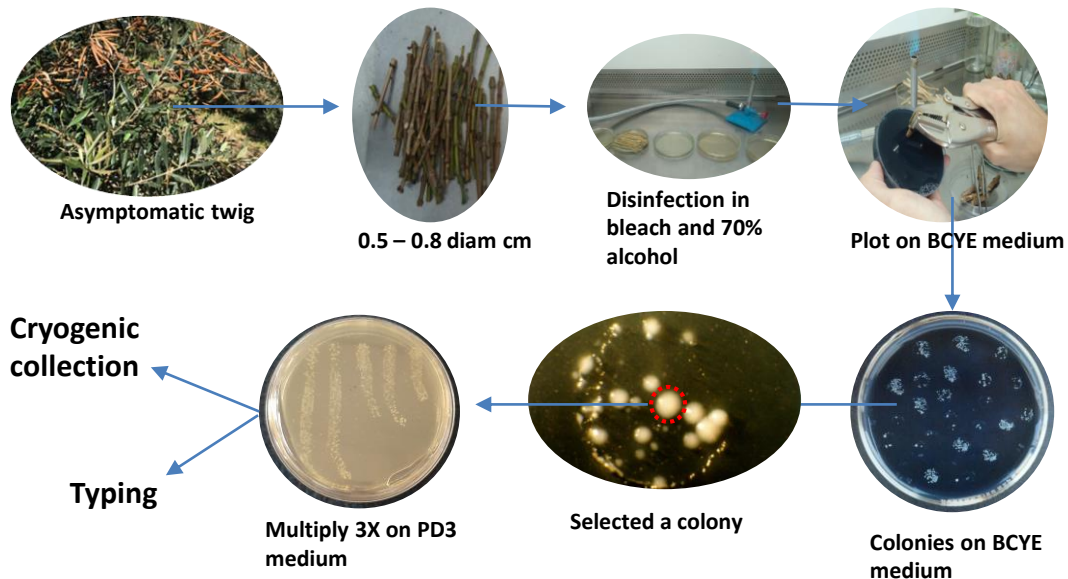
Methods

Collection of *X. fastidiosa* isolates

Origin (State/area)	No. plants	No. isolates	Altitude (m)
MINAS GERAIS			
MGA1 ^a	18	36	1300
MGA2	5	17	1310
MGA3	4	8	1318
MGA4	2	7	1329
MGA5	3	13	1235
MGA7	2	5	1400
MGA10	2	7	1780
MG13	5	19	1050
MGA14	2	5	1065
MGA17	2	5	883
MGA18	3	9	1329
MGA19	2	5	928
SÃO PAULO			
SPA1 ^a	4	14	1602
SPA2	3	13	690
SPA5	1	5	755
SPA6	2	9	883
TOTAL:	60	177	

^aMG - Minas Gerais State; SP - São Paulo State

Workflow for *X. fastidiosa* isolation



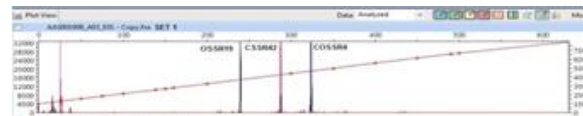
Methods

Setup a multiplexed of 12 SSR loci (PCR)

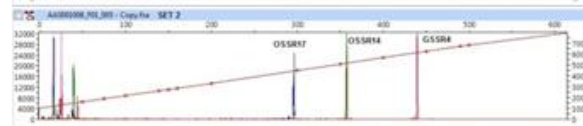
Set ¹	Primer ²	Dye ³	Forward sequence(5'-3') /Reverse sequence(5'-3')	Motif ⁴	concentration (μM) ⁵
1	COSSR4	FAM	CAAGGTGACCGTAGCCTAT/G CTGTCATTGGGTGATGC	(CAATACAC)	0.1
	CSSR42	PET	ATTACGCTGATTGGCTGCAT/GT TTCATTACGGGAACAC	(TGTTATC)	0.2
	OSSR19	NED	GCTGTGAACCTCCATCAATCC/G CAAGTAGGGTAAATGTGAC	(CAGGATCA)	0.2
2	OSSR14	VIC	GGCGTAACGGAGGAAACG/ATG AACACCCGTACCTGG	(TGATCCATCCCT GTG)	0.27
	OSSR17	FAM	AGTACAGCGAACAGGCATTG/A GCAACCAGGACGGGAAC	(TGCCTG)	0.27
	GSSR4	PET	GCGTTACTGGCGACAAC/GCT CGTTCCTGACCTGTG	(ATCC)	0.27
3	COSSR6	PET	TGCTGCGCGATAACCAAGT/GC ATCCAATCAGCCCTAACCT	(GTGATGCG)	0.27
	GSSR12	VIC	TTACGCTGATTGGCTGCATTG/G TCAAACACTGCCTATAGAGCG	(TATCTGT)	0.2
	COSSR1	FAM	GAAACAAGATGGCGGTTC/CA TTTAAACGGCGGCATA	(ATTGCTG)	0.27
4	COSSR3	FAM	AAGTATTCGCTACGCTACGC/GT GTGTTATGTGTGCCATTCTG	(CTGATGTG)	0.38
	CSSR18	NED	GTGCTTCAGAAAGTTGTG/GACT GTCTCTCTGTTTCAG	(GCCAA)	0.38
	COSSR5	VIC	ACACTGACACAACAGCCACCA/ AATGGTGGGTGTGATGGTTTC	(CATACAGA)	0.27

Capillary electrophoresis

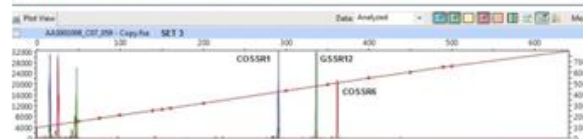
Set 1



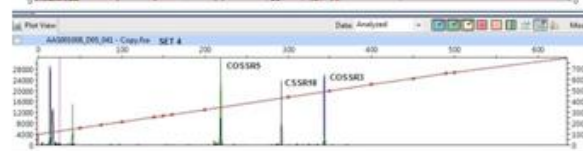
Set 2



Set 3



Set 4



Representative electropherograms after capillary electrophoresis separation of the fragments amplified by 3 multiplex PCR.

PeakScanner software

Methods

Overall statistics

General genetic information: (i) number of alleles per locus, (ii) allelic patterns, (iii) number of genotypes by population, (iv) Genetic diversity- software GenAlex (Peakal & Smouse, 2012).

Principal Coordinates Analysis (PCoA): using genetic distance among all **individuals** - GenAlex software

Number of genetic cluster: inferred by Bayesian statistic using Structure version 2.3 (Pritchard et al. 2000). Admix model with K ranging from 1 to 15.

Results

Genotypic and genetic diversity of *Xylella fastidiosa* populations isolated from olive trees in different geographic areas of São Paulo and Minas Gerais (Brazil).

INDEX	Populations															
	MGA1	MGA2	MGA3	MGA4	MGA5	MGA7	MGA13	MGA14	MGA17	MGA18	MGA19	SPA1	SPA2	SPA5	SPA6	Overall
No. individuos	36	17	8	7	13	5	19	5	5	9	5	14	13	5	9	177
No. Alleles	45	22	21	13	21	23	21	12	13	29	12	35	18	12	15	312
Gen. diversity	0.34	0.21	0.24	0.04	0.29	0.52	0.20	0.00	0.03	0.53	0.00	0.50	0.10	0.00	0.10	0.19
No. Genot.	22	5	4	2	6	3	6	1	2	5	1	7	4	1	3	72

No. individuals: number of total individuals sampled

No. Alleles: No. of different alleles

Gen. diversity: H_{Nei} unbiased genetic diversity corrected by the number of individuals in the population

No. Genot: No. of different genotypes

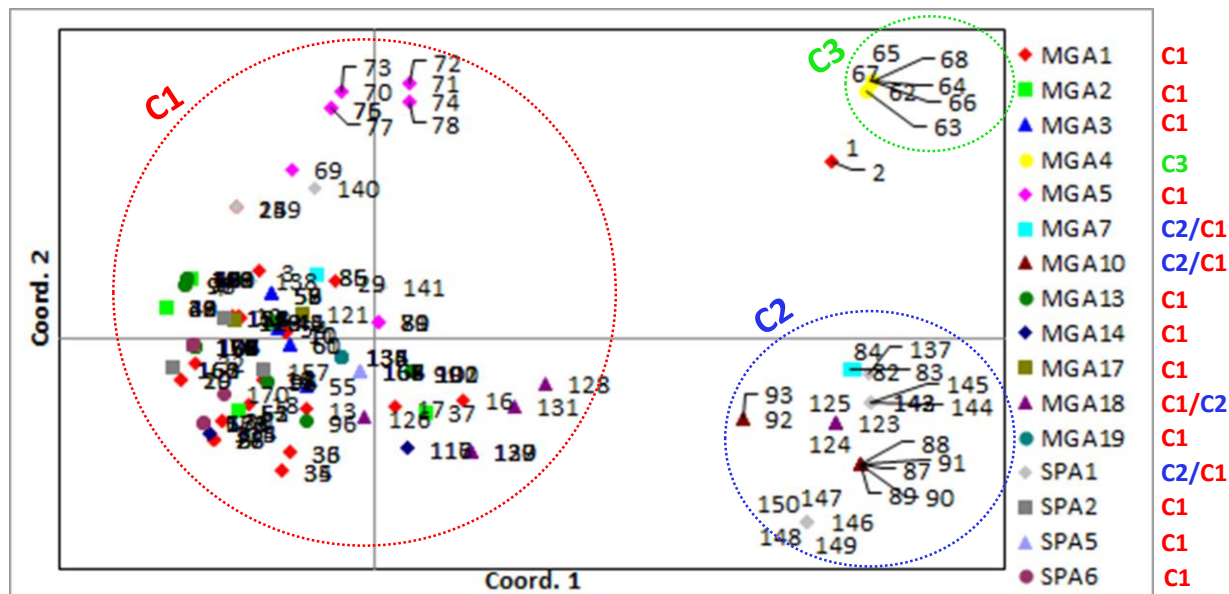
Gen. diversity of Xf populations;

- from CVC: $H_{Nei} = 0.46$ (Coletta-Filho et al, 2014)

- from CLS: $H_{Nei} = 0.60$ (Francisco et al, 2017)

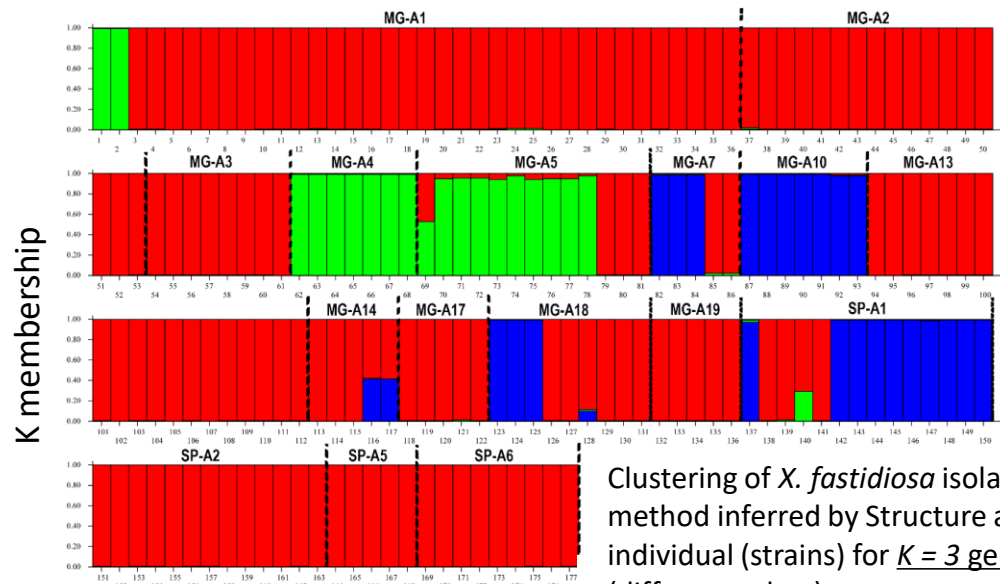
Results

Principal Coordinates Analysis (PCoA): using genetic distance among all individuals





Results



Clustering of *X. fastidiosa* isolates using Bayesian method inferred by Structure assignment of individual (strains) for $K = 3$ genetic groups (different colors).

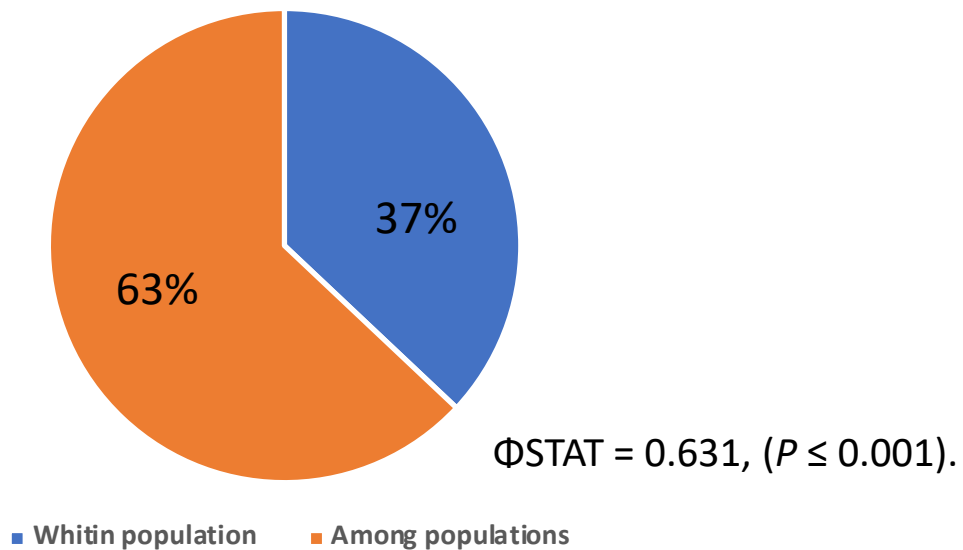
Similar clustering
obtained by PCoA

- C1
- C2
- C3 Except for MGA5



Results

AMOVA analysis of the total genetic diversity



Conclusion

- The low genetic diversity index, in general $H_{Nei} = 0.19$, could be explained by the recent outbreak of *X. fastidiosa* causing olive quick decline.
- So far, local characteristics such as natural reservoir or specific environment have no influence on genetic structuring of *X. fastidiosa* (few exceptions). Populations from 17 geographic regions were clustered into 3 genetic clusters.
- but, clonal dissemination of bacteria by infected vegetative material could be discarded.



Thanks for your attention

Acknowledgments:



Special thanks for the olive farmers by possibility of sampling material in their properties.

MsC Stud

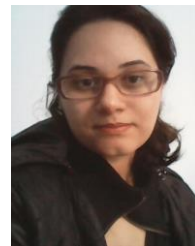


Nágela



UNIVERSIDADE FEDERAL DE SÃO CARLOS
CENTRO DE CIÊNCIAS AGRÁRIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM
PRODUÇÃO VEGETAL E BIOPROCESSOS ASSOCIADOS

MsC - Tech



Kelly

DISTRIBUTION AND GENETIC DIVERSITY OF *Xylella fastidiosa* ASSOCIATED
WITH OLIVE QUICK DECLINE IN SOUTHEASTERN BRAZIL

NÁGELA GOMES SAFADY

Araras
2019