

# A quick and efficient method for detection of *X. fastidiosa* in olive plants based on tissue-print

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## Introduction

### The Brazilian olive oil industry

✓ Young trees (<20 y old)



✓ Growers with few  
experience with the crop





## Introduction

### The Brazilian olive oil industry

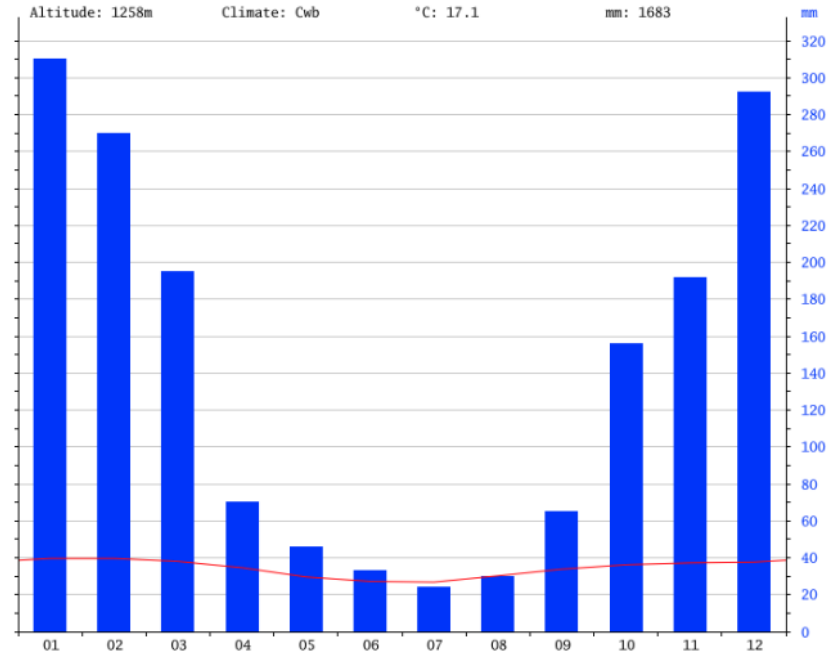
#### ✓ Cultivated at high altitudes

>700 m at sea level

↓ Temp. for flowering

↑ Humidity

1683 mm



Rainfall distribution over the year (mm)



# The Brazilian olive oil industry

## ✓ Others Disorders

Biotic and Abiotic







## OQD symptoms!



## OQD symptoms?



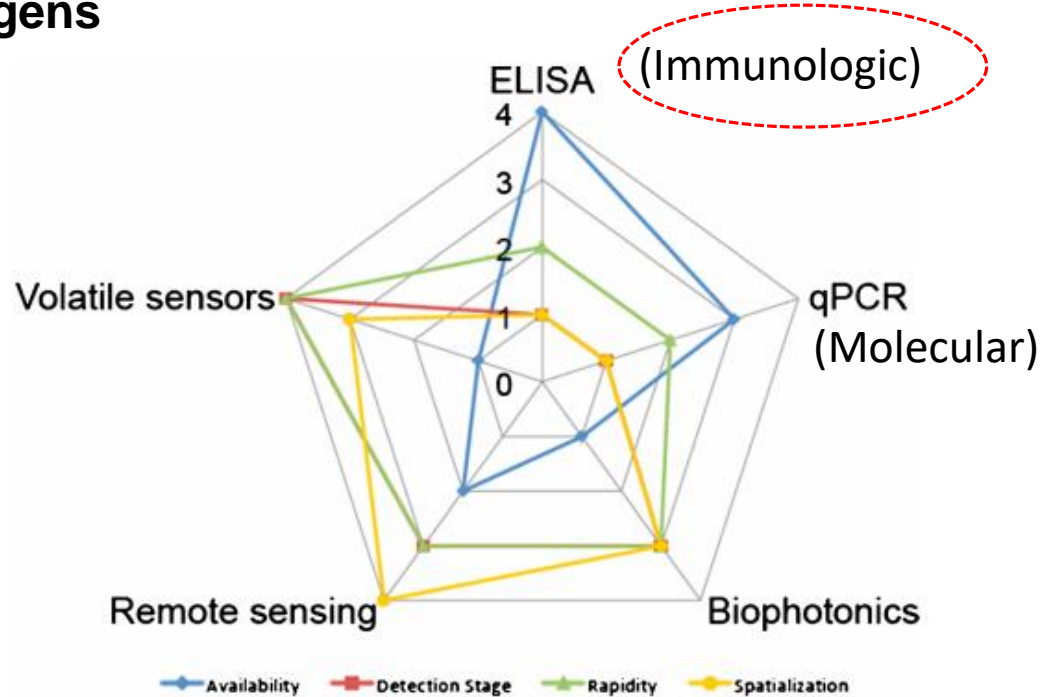
## Objective

To help growers to discern about leaf desiccation symptoms relate to Xf infection from other problems by diagnosis of bacteria in suspicious plants.



## Methods

### Diagnosis of plant pathogens



Adapted from Martineli et al., 2015

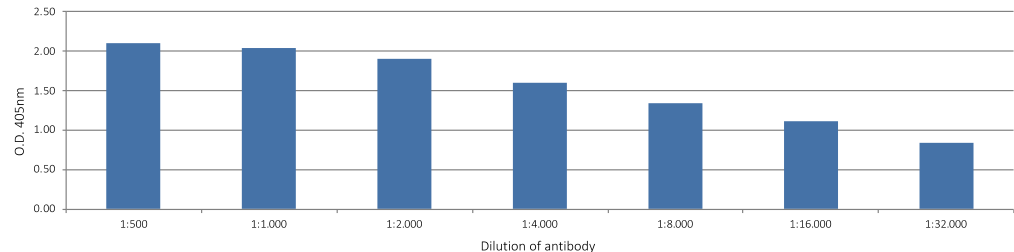
## Methods

✓ Polyclonal antibody raised in rabbit using a mix of *X. fastidiosa* subsp. *pauca* STs provide by us.

<http://www.rheabiotech.com.br/pt-br/anticorpos-primarios-policonais>



✓ Reactivity of antibody against antigen by ELISA





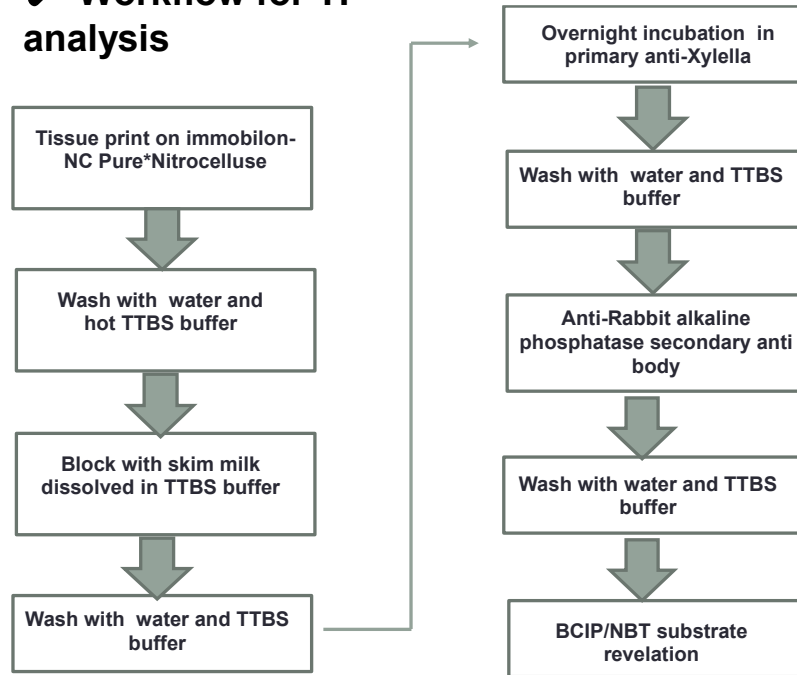


## Methods

✓ Tissue print strategy was used for fixed *X. fastidiosa* on Nitrocelulose pure membrane by printing olive twigs (0.5 – 0.8 cm diameter)



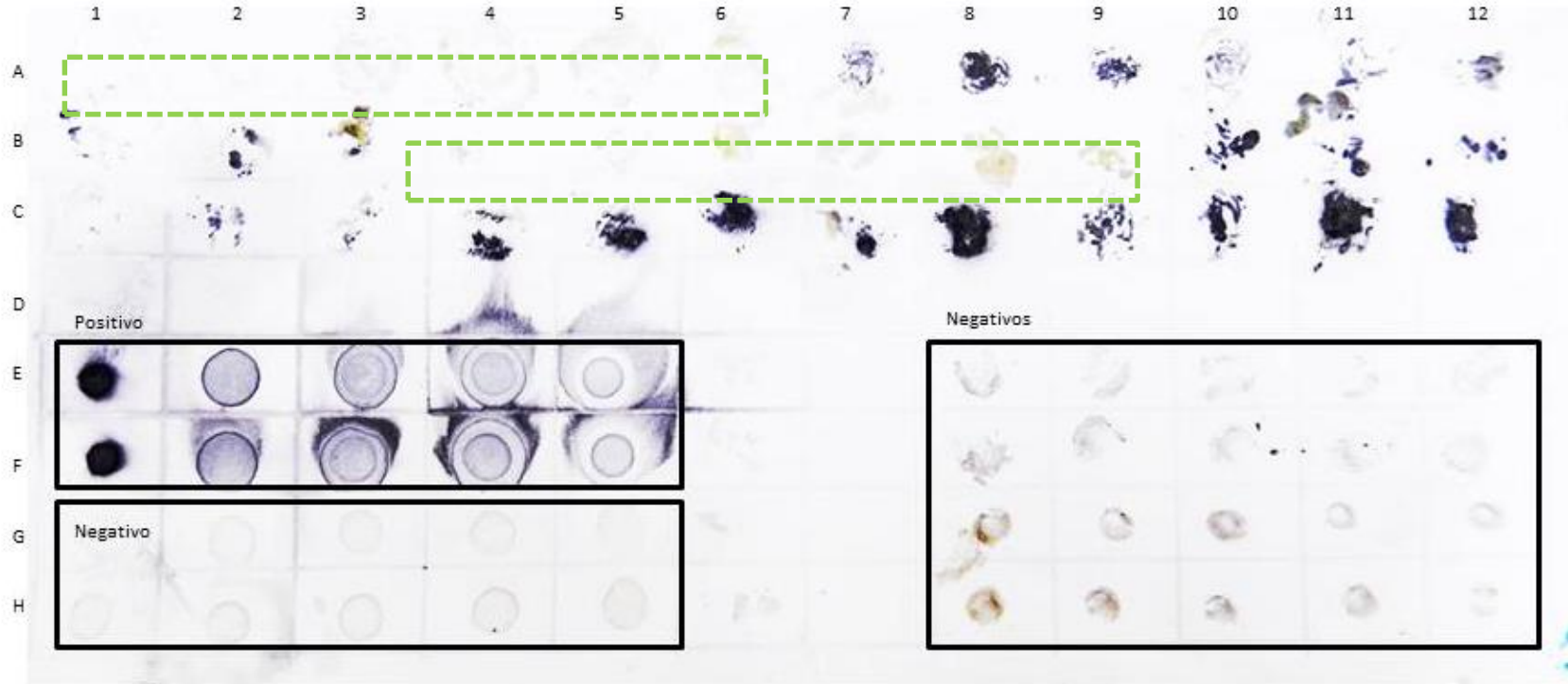
### ✓ Workflow for TP analysis





## Results

✓ NC membrane printed with **health** and symptomatic samples

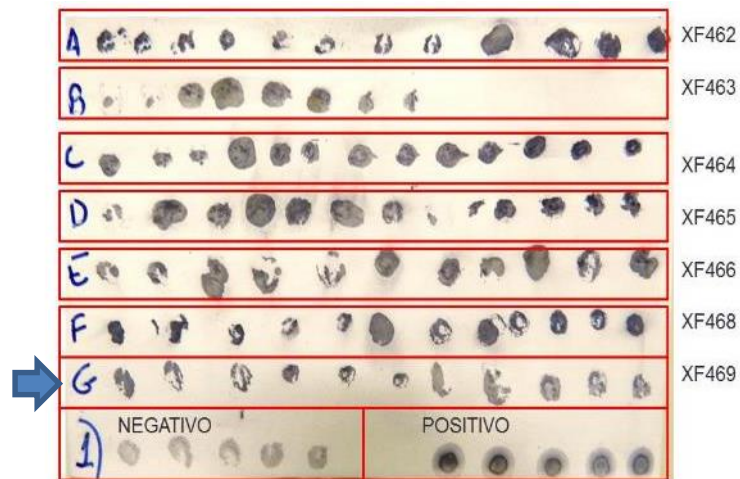


## Results

### ✓ Comparative analysis: conv PCR<sup>1</sup> x qPCR<sup>2</sup> x TP

Conv.PCR<TP<qPCR

Samples	Conv PCR	qPCR	TP	Samples	PCR conv	qPCR	TP
XF418	Pos	20.40	Pos	XF 471	Neg	22.04	Pos
XF419	Neg	32.28	Pos	XF 472	Pos	22.58	Pos
XF421	Pos	15.95	Pos	XF 473	Neg	20.35	Neg
XF422	Neg	17.2	Pos	XF474	Neg	20.42	Pos
XF 457	Neg	32.46	Neg	XF475	Pos	21.47	Pos
XF 458	Neg	31.46	Neg	XF476	Pos	18.55	Pos
XF 459	Neg	27.29	Pos	XF477	Pos	23.4	Pos
XF 460	Neg	19.76	Pos	XF478	Pos	26.47	Pos
XF 461	Neg	29.36	Pos	XF479	Pos	19.48	Pos
XF 462	Neg	26.56	Pos	XF480	Pos	21.68	Pos
XF 463	Neg	23.72	Pos	XF481	Neg	26.68	Pos
XF 464	Neg	22.06	Pos	XF482	Pos	21.36	Neg
XF 465	Neg	31.7	Pos	XF483	Neg	25.20	Neg
XF 466	Neg	22.28	Pos	XF484	Neg	23.20	Neg
XF 468	Neg	20.55	Pos	XF485	Pos	23.52	Pos
XF 469	Neg	und	Pos	XF486	Neg	26.40	Pos
XF 470	Neg	23.85	Pos	XF487	Neg	Und	Pos

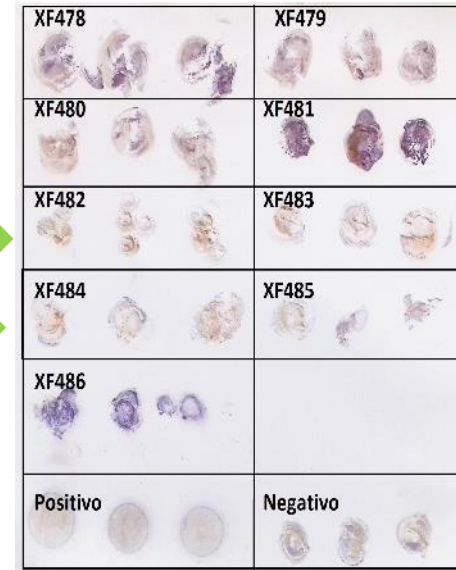


<sup>1,2</sup>CTAB-based DNA ext protocol  
<sup>1</sup>RST31/33 primers  
<sup>2</sup>Oliveira et al. 2002 (CVC specific primers)

## Results

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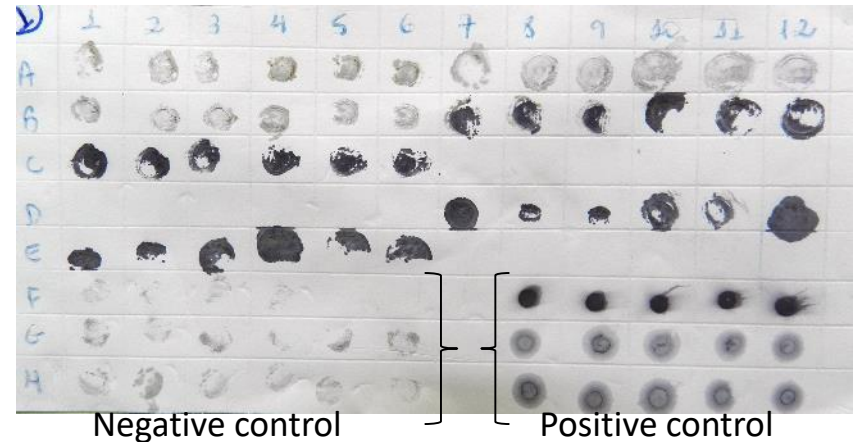
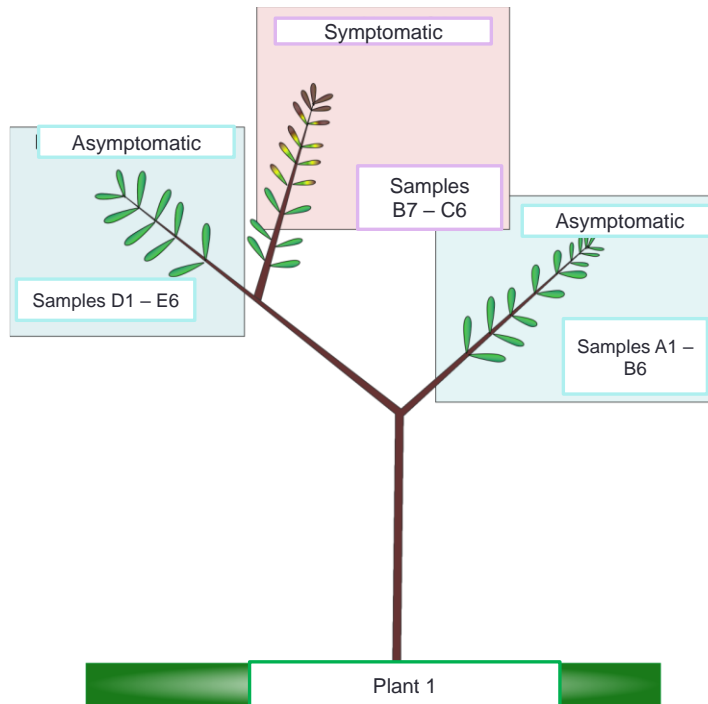


1,2CTAB-based DNA ext protocol  
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## Results

### ✓ Validation TP protocol: tracking XF in plant





## Results

### ✓ Training workers' farms for printing the cards



Cards mailed to the lab



Feed back of results

Envelope #	Origin	Sample ID	Tissue print	Illustration
1	COIVARAS, Dona Ligia 21.08.18, Aiuruoca- MG	1A Arbequina	Positivo	
		1B koroneiki	Positivo	
		1C Arbequina	Negativo	
		1D Arbosana	Negativo	
		1E Arbosana	Positivo	
		1F koroneiki	Positivo	
		2A Arbequina 2,5 anos	Talvez Positivo	
		2B Arbequina 2,5 anos	Negativo	
		2C Arbosana 2,5 anos	Negativo	
		2D Arbosana 2,5 anos	Negativo	
2E Arbosana 2,5 anos	Negativo			
2	Fazenda Guapiara, Aiuruoca- MG 31.08.2018	5A Ascolano 8 anos	Positivo	
	5B Ascolano 8 anos	Positivo		
	5C Ascolano 8 anos	Positivo		
	Fazenda Boa Vista, Aiuruoca- MG 31.08.2018	5D Ascolano 8 anos	Positivo	
	5E Ascolano 8 anos	Positivo		
	5F Ascolano 8 anos	Positivo		
	Reserva do Sauá, Aiuruoca - MG 30.08.2018	4A koroneki 8 anos	Negativo	
		4B koroneki 8 anos	Positivo	
		4C koroneki 8 anos	Positivo	
		4D koroneki 8 anos	Positivo	
4E koroneki 8 anos		Negativo		
4F koroneki 8 anos		Positivo		
3	O Lavandário, Cunha - SP 01.09.2018	1A Arbequina	Negativo	
		1B Arbequina	Positivo	
		1C Koroneiki	Negativo	
		1D Koroneiki	Negativo	

**TP  
analysis  
and  
interpret  
ation**

## Conclusion

- This simple TP protocol has been help us on diagnosis of XF in olive plants with suspicious OQD symptoms.



## Thanks for your attention

### Acknowledgments:



2016/02176-7

Und grad stud  
Emile



MsC Stud



Nágela

MsC - Tech



Kelly