

# Diseases induced by *X. fastidiosa* subsp. *pauca*: ecology, epidemiology and management

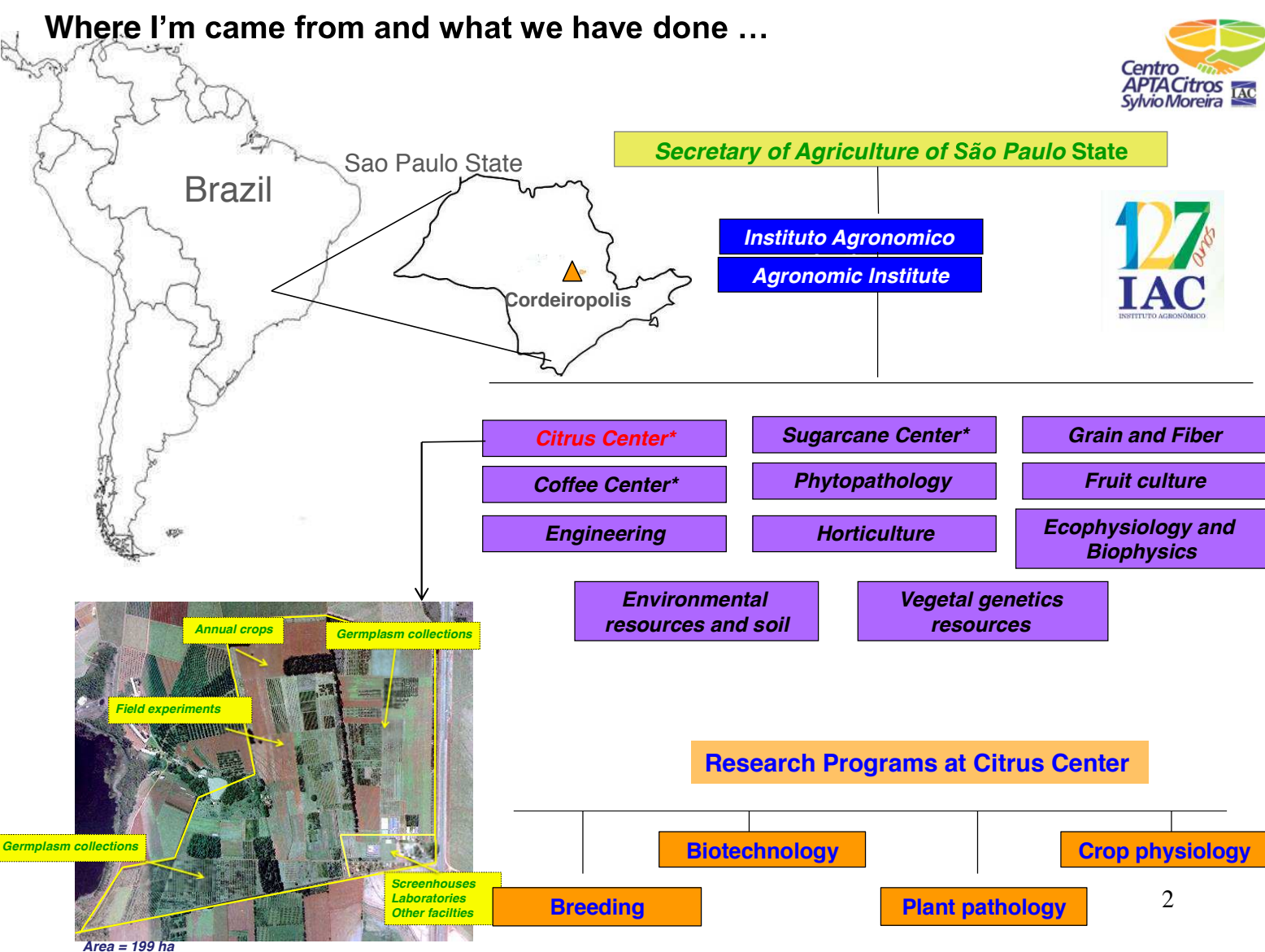
**Helvécio Della Coletta Filho**

**Citrus Research Center / Agronomic Institute / São Paulo  
State / Brazil**

**helvecio@centrodecitricultura.br**

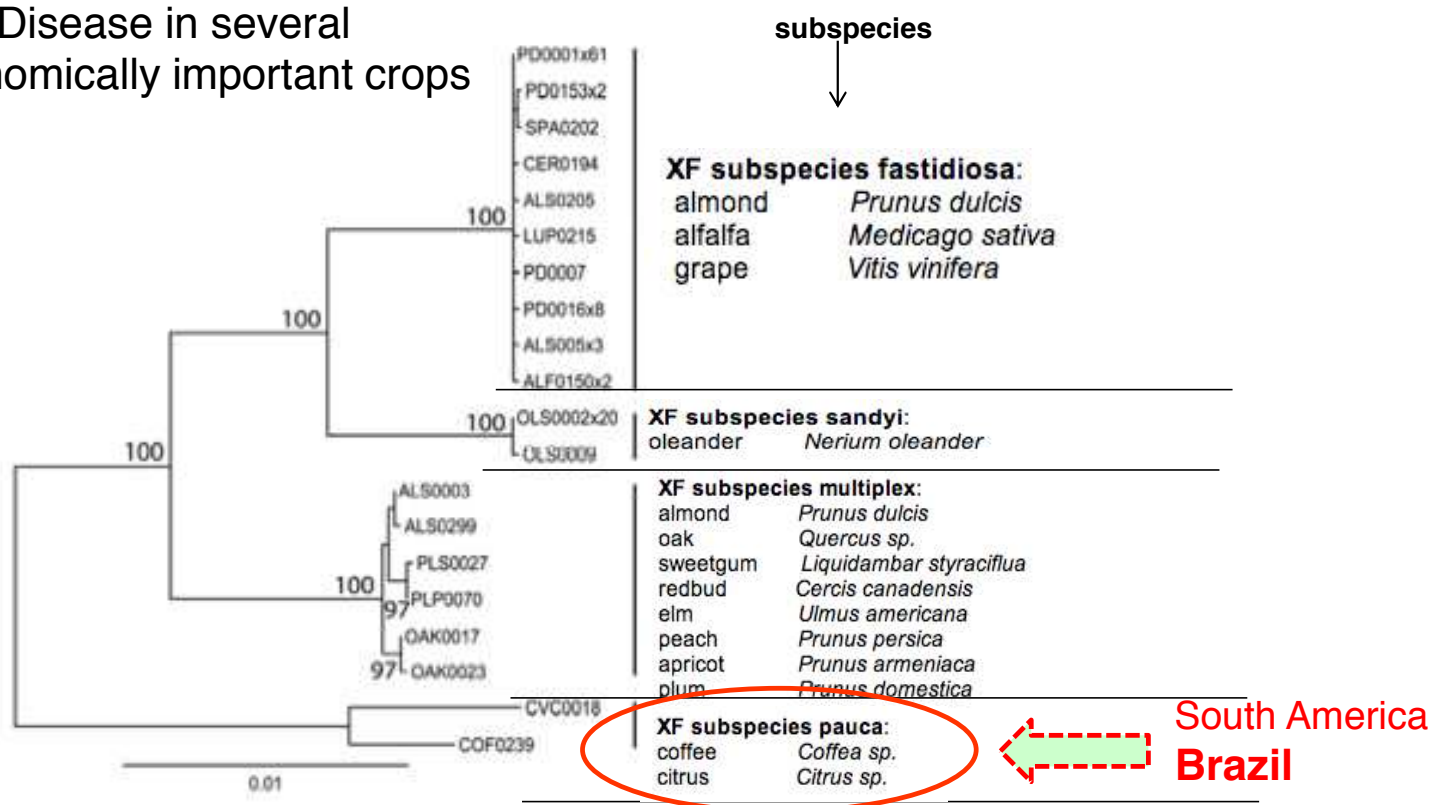


# Where I'm came from and what we have done ...



✓ Infection a hundred of plants species

✓ Disease in several economically important crops



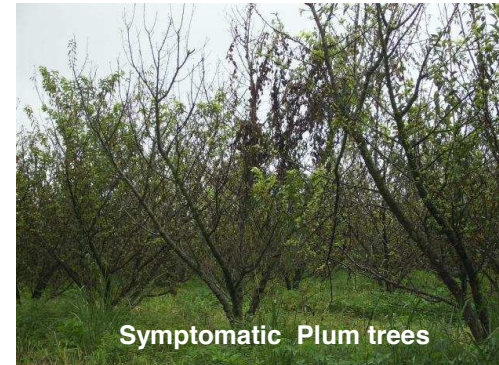
Maximum likelihood phylogenetic tree of *Xylella fastidiosa*  
Yuan et al., 2010      Phytopathology 100:601-611.

## ***X. fastidiosa* in Brazil**

### ***X. fastidiosa* subsp. *multiplex***

causing Plum Leaf Scald

- Firstly reported in neighbor country (AR) in 1935



- in Brazil in 1978 in the Southern region

French & Kitajima. Plant Disease, 1978

Nowadays is spread for all regions that produced plum (*Prunus domestica*) in Brazil





***X. fastidiosa* subsp. *pauca***

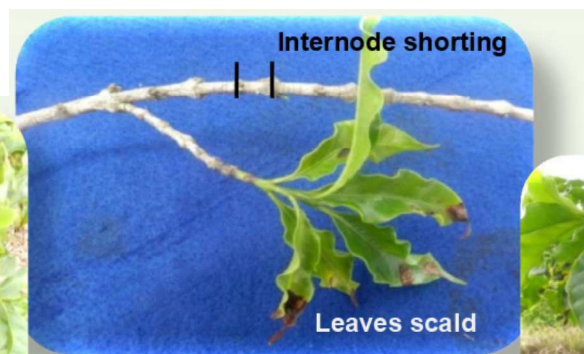
**Citrus Variegated Chlorosis - CVC**

(Rosetti et al., 1990 )



**Coffee leaf scorch and/or branch atrophy**

(Paradela-Filho et al., 1995 e Lima et al., 1998)

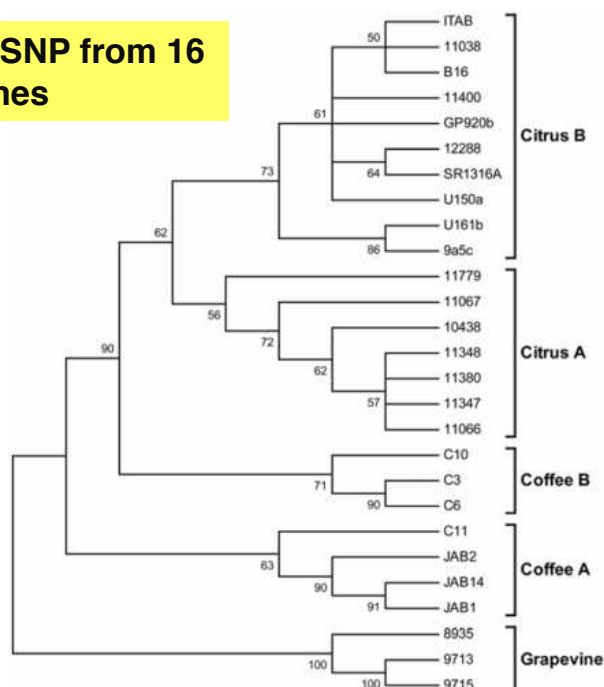


## *X. fastidiosa* subsp. *pauca*

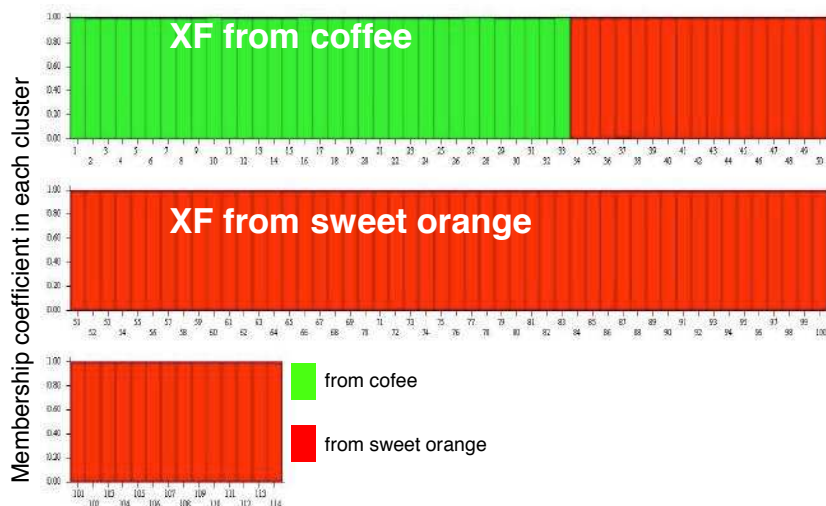
from sweet orange  
from coffee

**Genetically different !**

by SNP from 16  
genes



by Microsatellites markers  
from 12 loci



Genetic relationship of *X. fastidiosa* by  
MEGA using isolates from ad hoc  
collection.

(Wickert et al., 2003 – Phytopathology)

Genetic relationship of *X. fastidiosa* by STRUCTURE  
using isolates from sweet orange and coffee plants  
closely sampled .

(Francisco, 2014– submitted)

*X. fastidiosa subsp. pauca*

from sweet orange  
from coffee

Biologically different !

Patterns of sweet orange and coffee plants infection and colonization buy *X. fastidiosa* subesp. *pauca* isolates from:

		Ratio for infection and symptoms in:			
XF from	Isolate	Citrus host		Coffee host	
		Infection <sup>a</sup>	CVC symptoms <sup>a</sup>	Infection <sup>b</sup>	CLS symptoms <sup>a,b</sup>
Citrus					
1	10	1/2 (6.1)	1/2	0/20	0/20
1	11	4/13 (5.7 ± 0.5)	0/13	0/20	0/20
1	35	9/11 (6.3 ± 0.1)	1/11	0/20	0/20
1	36	9/19 (4.9 ± 0.3)	1/19	0/20	0/20
1	37	8/15 (5.4 ± 0.3)	3/15	0/20	0/20
1	6570	8/11 (5.4 ± 0.3)	1/11	0/20	0/20
2	18	3/6 (6.1 ± 0.4)	3/6	0/20	0/20
Coffee					
3	29	0/14	0/14	7/20 (4.9 ± 0.2)	0/20
4	1	0/13	0/13	18/20 (5.2 ± 0.1)	0/20
4	4	0/16	0/16	12/20 (5.1 ± 0.1)	0/20
4	3124	0/16	0/16	6/20 (4.8 ± 0.2)	0/20
5	32	0/13	0/13	11/20 (5.4 ± 0.2)	0/20
7	8	0/15	0/15	16/20 (4.9 ± 0.1)	0/20
7	24	0/8	0/8	14/20 (4.7 ± 0.2)	0/20
7	33	0/7	0/7	11/20 (5.4 ± 0.1)	0/20

Adapted from Almeida et al., 2008 - AEM

*X. fastidiosa subsp. pauca*

from sweet orange causing the CVC

from coffee

Timeline of CVC in Brazil

1987 1989 1990 etiology 1993

Frist reported



Grafting transmission of CVC shown an **biotic agent** associated!

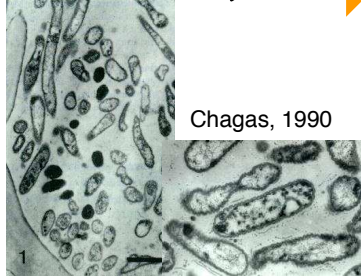


from disease



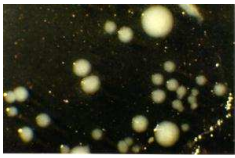
health

Microscopy showed *X. fastidiosa* bacterium-like in the xylem vessels of infected plants!



Chagas, 1990

*X. fastidiosa* isolation from disease leaves!



**Koch's postulate!**

Chang et al., 1993



Hypothesis:

- . Nutritional deficiency  
Zn, B, K
- . Virus disease



## Timeline of CVC in Brazil and actions



### CVC spread information

#### ✓ Vector transmission

Species of Sharpshooters

Lopes et al., 1996.



#### ✓ Diagnosis

Previous to disease symptoms expression.

Pooler & Hartung, 1995

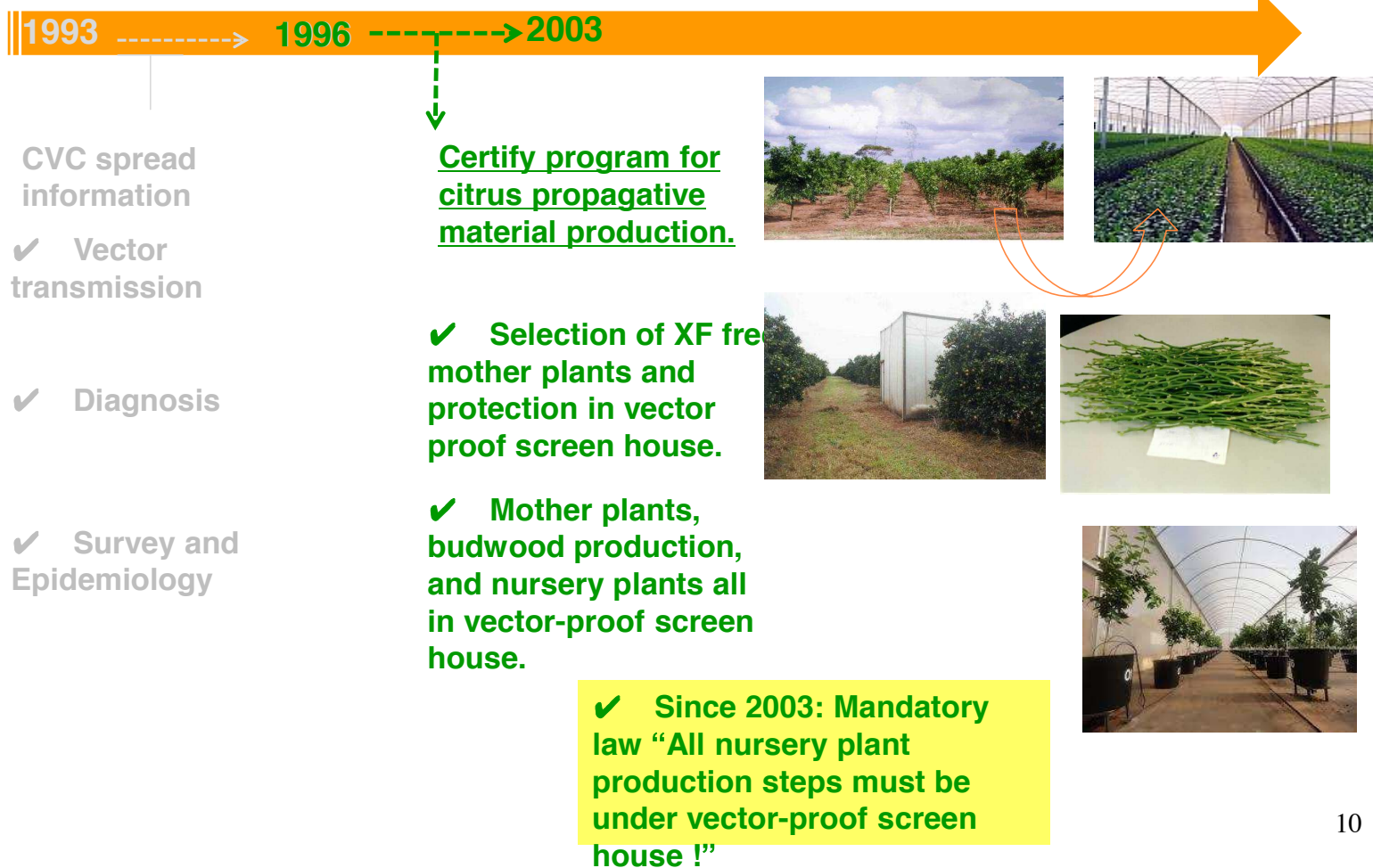
#### ✓ Survey and Epidemiology

How far the disease was spread from the first reported spot.

The importance of inoculum source from outside and from inside the orchards.

Laranjeira, 1993

## Timeline of CVC in Brazil and actions



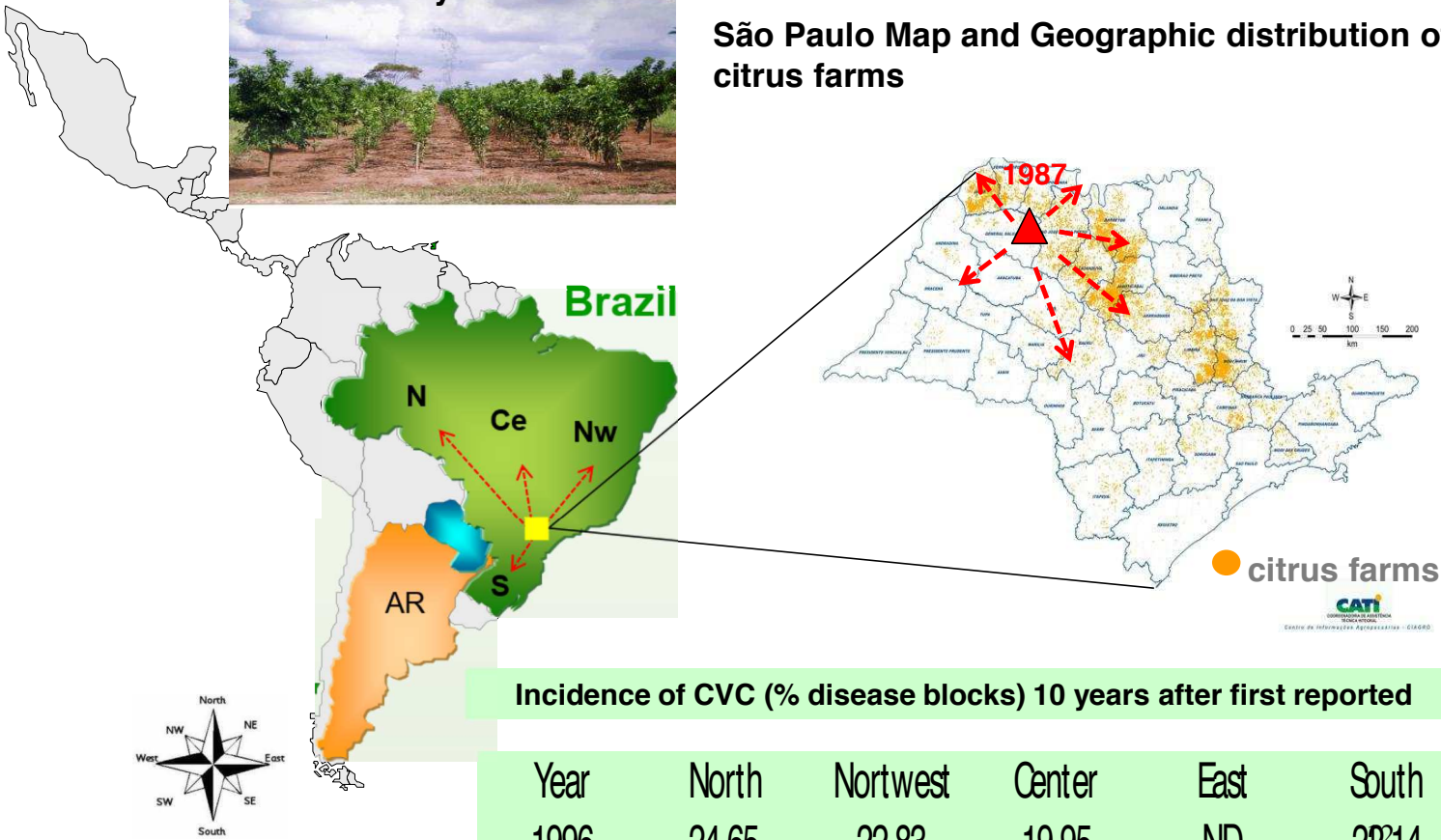
## Timeline of CVC in Brazil and actions



Origin and Geographic distribution of CVC



São Paulo Map and Geographic distribution of citrus farms





## Plant hosts and economic damage

### Hosts:



- all commercial sweet orange (*C. sinensis*) varieties are susceptible
- Most of mandarins (*C. reticulata*) varieties are resistant, but with some few exception (Carvalhais and Wilking).
- Most of tangors (*C. sinensis* x *C. reticulata*) are resistant, but with some few exception (Ortanique, Temple, Umatilla)
- All lemons, acid lime, and pummelos tested until now are resistant.

Alternative hosts: *Nicotiana tabacum* and *Cataranthus roseus*



## Plant hosts and economic damage

### Hosts:

- all commercial sweet orange varieties are susceptible, but **with some exceptions**.

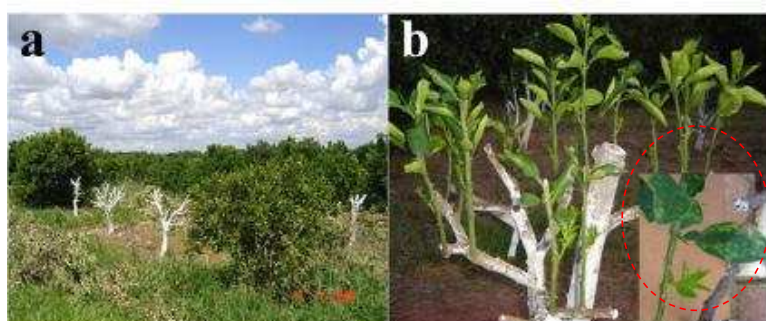


Navelina ISA 315: A cultivar resistant to citrus variegated chlorosis



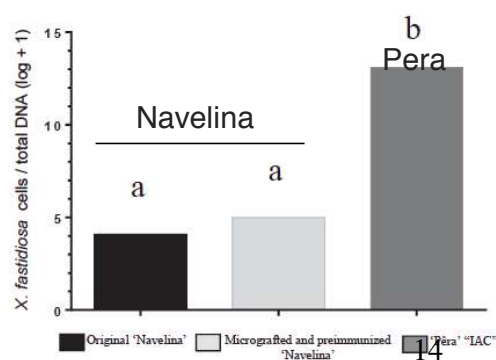
André Luiz Fadel <sup>a,\*</sup>, Eduardo Sanches Stuchi <sup>b</sup>, Sérgio Alves de Carvalho <sup>c</sup>,  
Maria Teresa Federici <sup>d</sup>, Helvecio Della Coletta-Filho <sup>c</sup>

Disease plants in field    Top grafting of Navelina  
on disease plant



Susceptible Pera variety

*X. fastidiosa* in tested plants



**Plant hosts and economic damage**



Reduction (%) on fruit production and juice quality on CVC disease plants compared to no-disease

Symptoms level	Fruit production		Juice quality	
	Weight	Number	SS*	Ratio
middle	16.5	13.9	-14%	-22%
strong	75	70.9		

adapted from Laranjeira, 2004

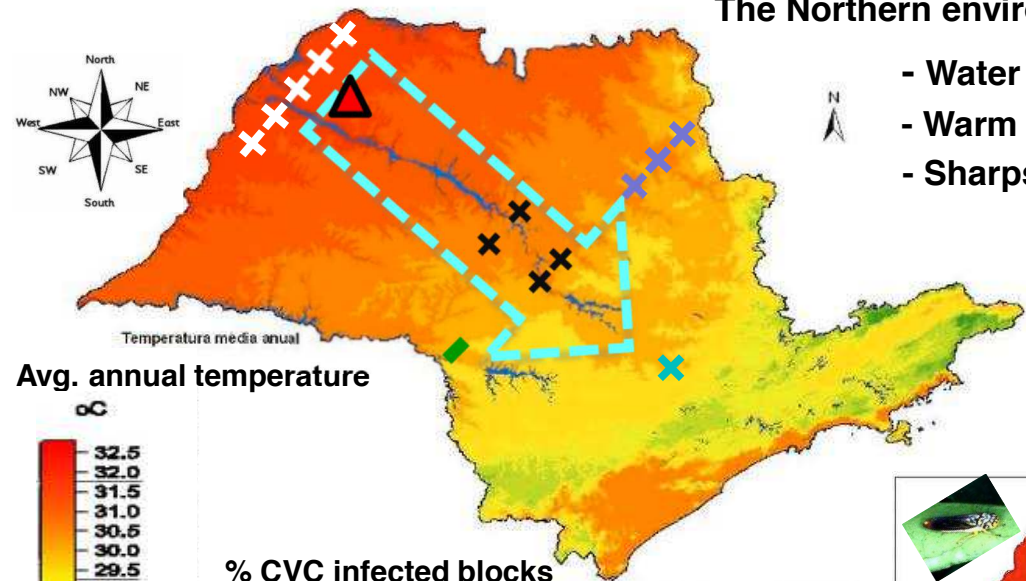
\*SS - total Soluble Solid - OBrix

Epidemiology: the spatial incidence and severity of CVC

CVC incidence through São Paulo State

The Northern environment

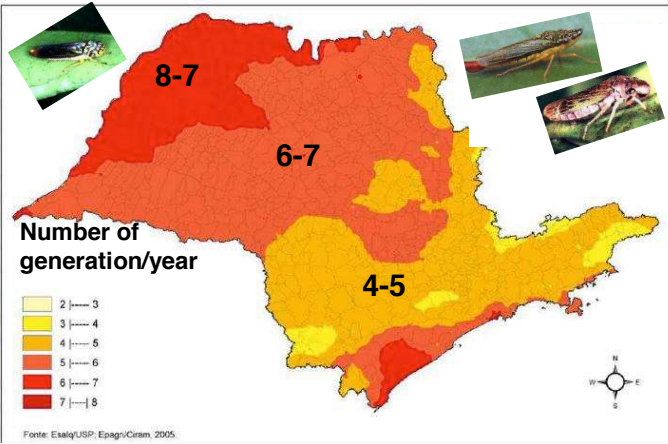
- Water stress
- Warm temperatures
- Sharpshooters population



% CVC infected blocks

	Center	North	Northwest	West	East	South
2009	52.65	52.52	46.71	1.15	28.23	1.57
2010	38.2	53.03	2.81	4.41	34.86	3.04
2011	42.37	59.73	52.55	1.06	45.15	2.05
2012	42.16	58.35	47.19	0	40.78	3.77

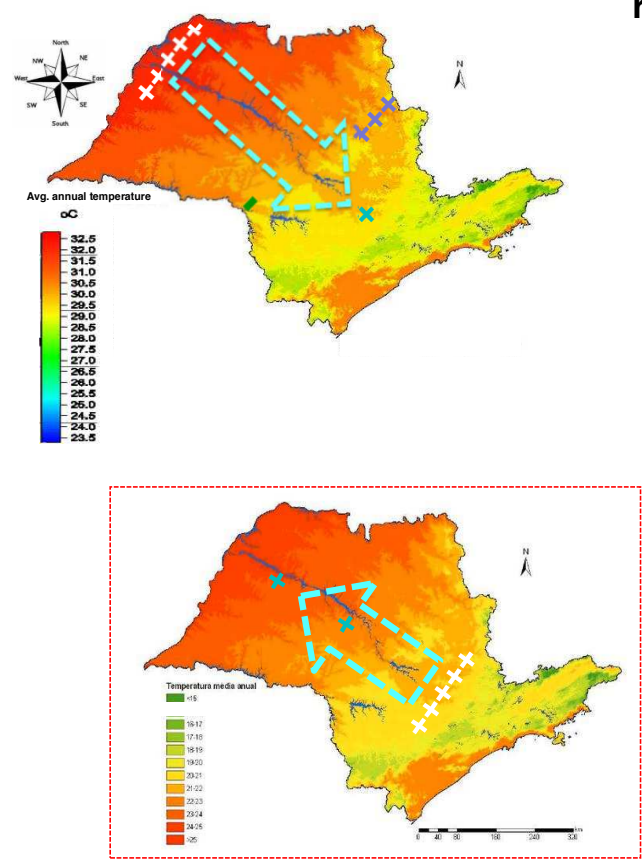
Adapted from Fundecitrus





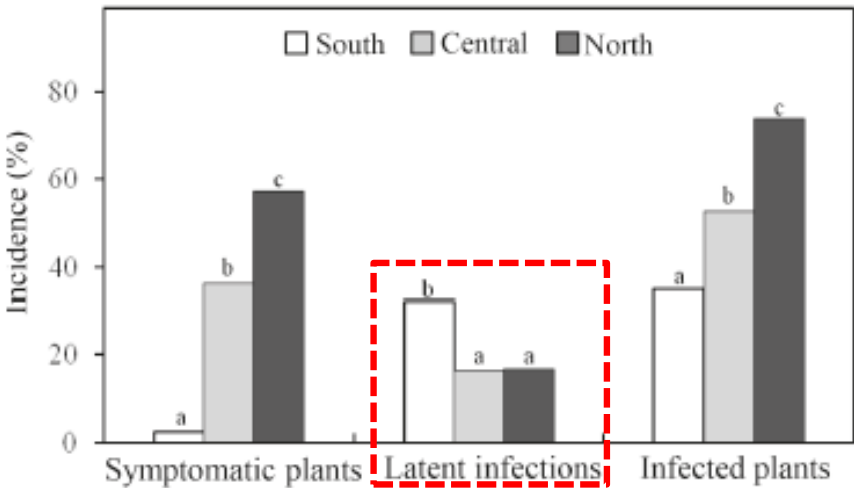
Epidemiology: the latent infection

CVC incidence through São Paulo State



*X. fastidiosa* - the latent infection is higher in South region

Journal of Plant Pathology (2013), 95 (3), 493-498

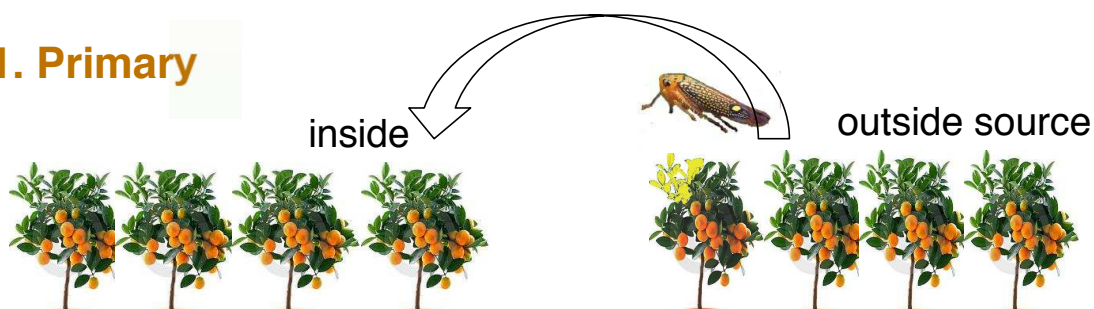


Coletta-Filho et al., 2013

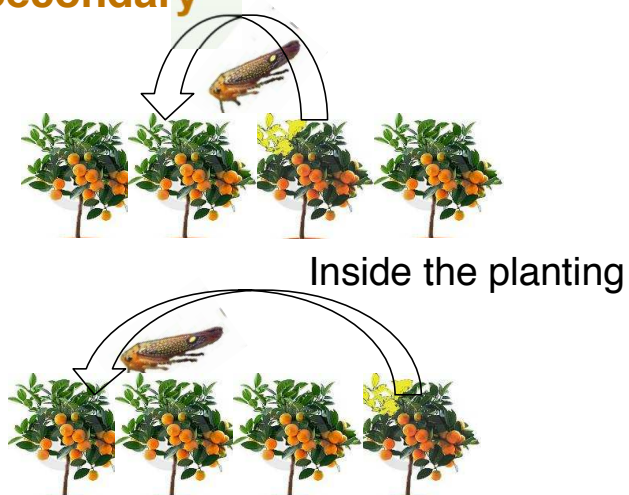
Both incidence  
and CVC severity are  
environmentally dependent!

Epidemiology: 1<sup>a</sup> and 2<sup>a</sup> spreads forms are important for CVC !

## 1. Primary



## 2. Secondary



Differently to PD pathosystem, for CVC limited information is available about others sources of inoculum outside sweet orange and its importance to disease epidemiology.

**Epidemiology: sources of inoculum**

Weeds plants present in orchards

Frequency of infection of weeds plants mechanically inoculated with the CVC strain of *Xylella fastidiosa*.

Scientific name	CVC strain		
	1st exp.	2nd exp.	3rd exp.
<i>Medicago sativa</i>	... <sup>a</sup>	1/10	5/10
<i>Echinochloa crus-galli</i>	8/10 <sup>b</sup>	6/10	7/10
<i>Brachiaria decumbens</i>	2/9	3/10	8/10
<i>Digitaria horizontalis</i>	3/10	1/10	0/10
<i>Brachiaria plantaginea</i>	3/9	9/10	9/10
<i>Solanum americanum</i>	2/9	4/10	3/10
<i>Bidens pilosa</i>	4/10	1/10	0/10
<i>Citrus sinensis</i> cv. Caipira	10/10	2/6	...



Family:  
Poceacea

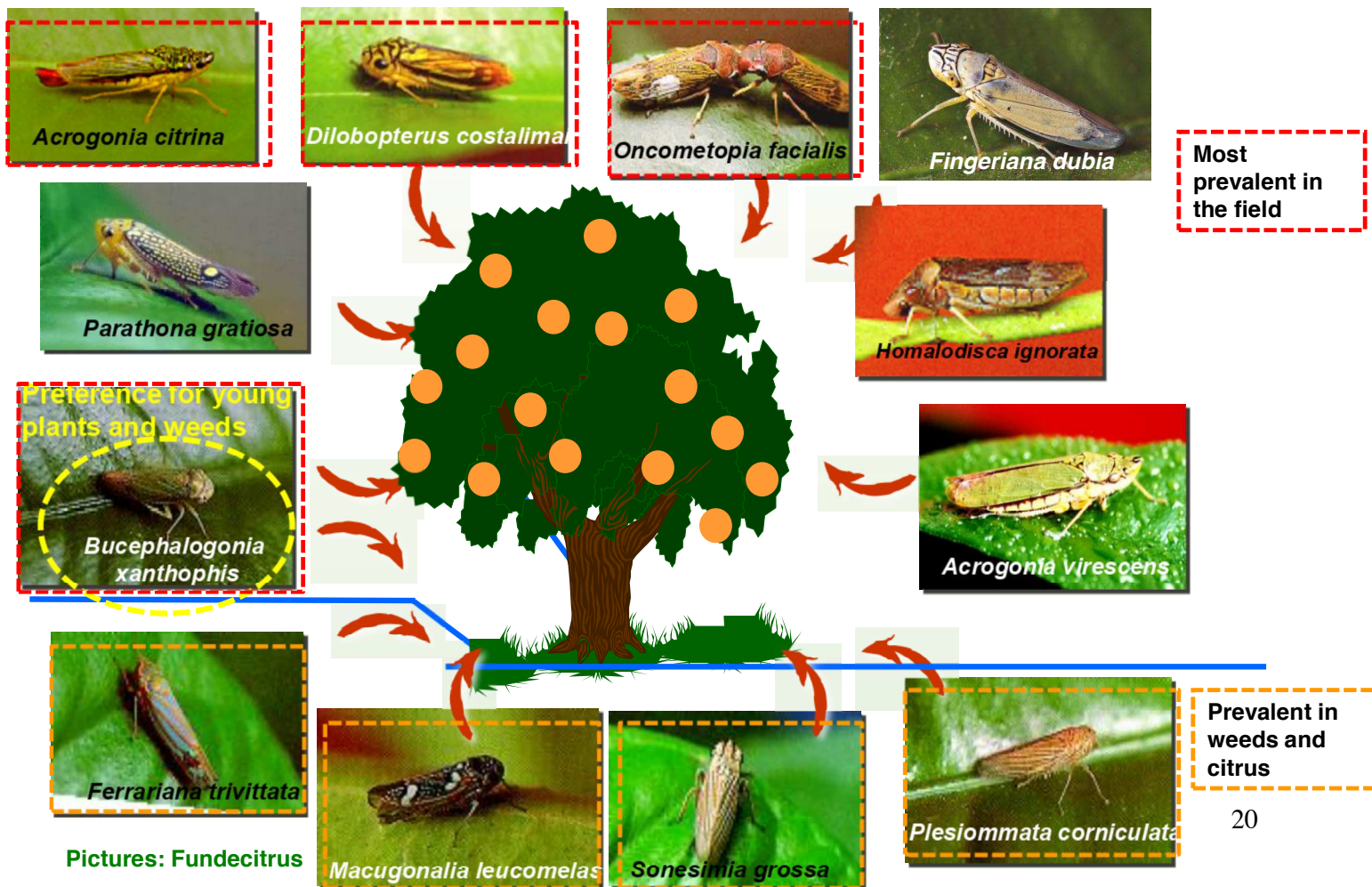


Plants were injected twice with suspensions containing  $10^8$  to  $10^9$  CFU of XF/ml and evaluated by PCR 60 DAI

Adapted from: Lopes et al. 2003. Plant Disease 87:544

**No acquisition and/or transmission assays were done.**

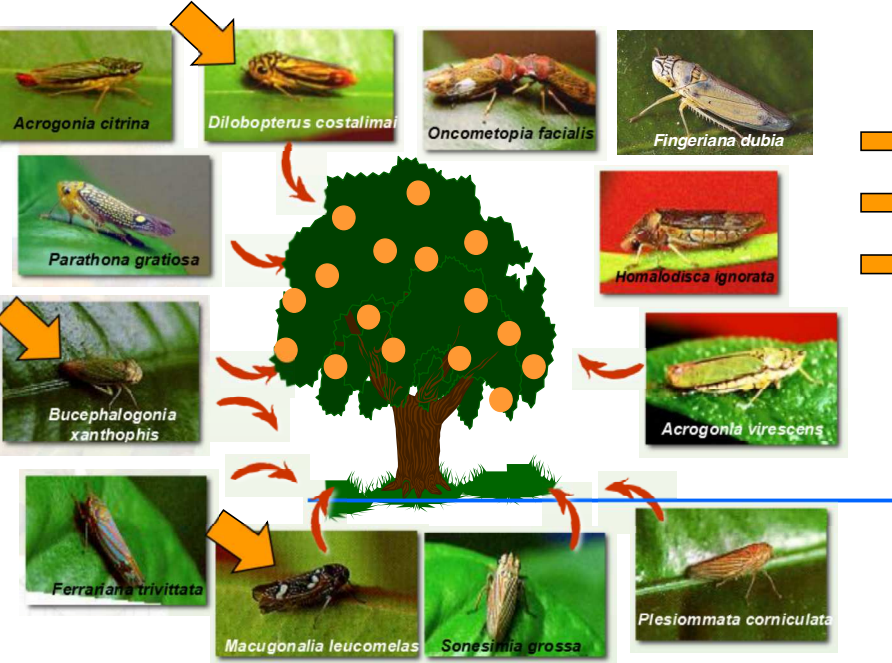
Epidemiology: 13 different species of sharpshooters as vectors





Epidemiology: vectors

Transmission efficiency is low and different among the species

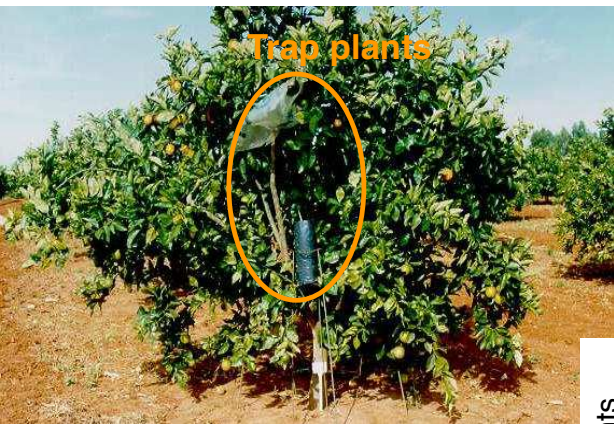


Pictures: Fundecitrus

Sharpshooter sps	Transmission efficiency %
Macugolania	17.30%
Bucephalogonia	12.80%
Dilobppterus	5.50%
Plesiommata	2.90%
Parathona	2.80%
Acrogonia	2.30%
Ferrariana	1.90%
Oncometopia	1.30%
Sonesimia	1.20%
Homalodisca	0.50%
A. virescens	0.30%

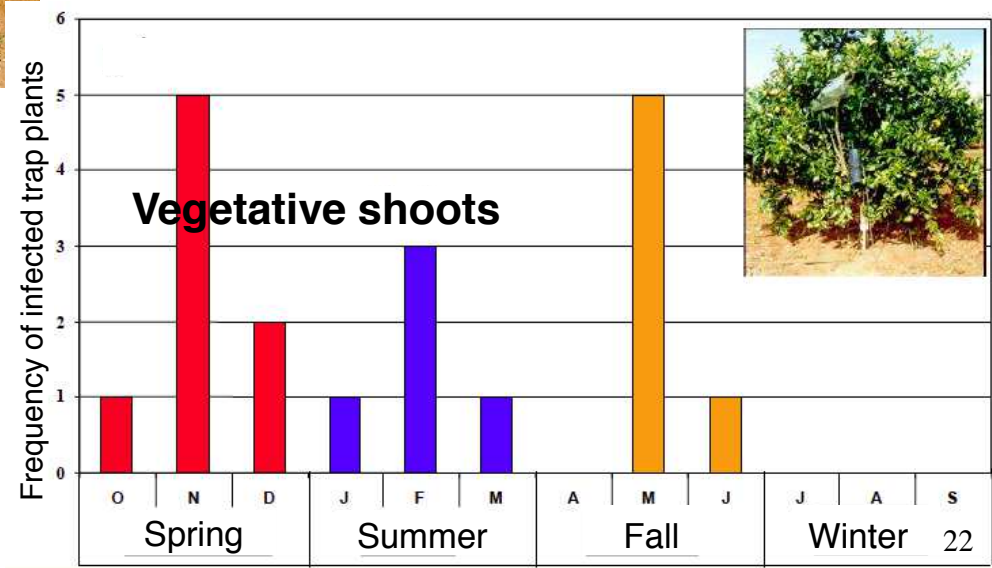
Adapted from: P Yamamoto

Epidemiology: natural infectivity of vectors and transmission



Successful transmission is higher in the wet and warm seasons.

Frequency of trap plants naturally infected by XF.  
Avg of three orchards.



Adapted from: JRS Lopes

## Epidemiology: faunistic distribution of sharpshooters

Different sites of the citrus block host different populations of sharpshooters.

Site	Total collected	No. of species	No. of collections	Shannon-Wiener Diversity ( $H'$ )
Forest edge	1012	14	58	1.39
Stand periphery	1003	17	58	1.30
Stand interior	846	13	56	1.45

Adapted from: Coelho et al, 2008

## Epidemiology: seeds transmission

- There is NO transmission of XF from seeds to seedlings

Seven years of negative detection results confirm that *Xylella fastidiosa*, the causal agent of CVC, is not transmitted from seeds to seedlings

Helvécio Della Coletta-Filho • Sérgio Alves Carvalho •  
Luis Fernando Carvalho Silva •  
Marcos Antonio Machado

Eur J Plant Pathol (2014) 139:593–596



Evaluation by PCR of *Xylella fastidiosa* subsp. *pauca* transmission through citrus seeds with special emphasis on lemons (*Citrus limon* (L.) Burm. f)

A.B. Cordeiro<sup>a</sup>, V.H. Sugahara<sup>a</sup>, B. Stein<sup>b</sup>,   , R.P. Leite Junior<sup>a</sup>

Crop Protection (2014) 62:86-92

 Open Access  Subscription Access

### LACK OF EVIDENCE FOR TRANSMISSION OF XYLELLA FASTIDIOSA FROM INFECTED SWEET ORANGE SEED

J.S. Hartung, S. Nian, S. Lopes, A.J. Ayres, R. Brialsky

doi: 10.4454/JPP.V96I3.011

J Plant Pathol (2014) doi: 10.4454/JPP.V96I3.011



CVC Management

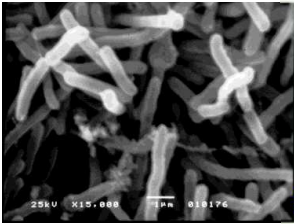
The tetrahedron of  
a vectored disease

- Environment favorable  
conditions
- ✓ Warm temperatures
  - ✓ Irregular rainfall  
distribution



Minimizing  
vector  
population

Bacterium virulence

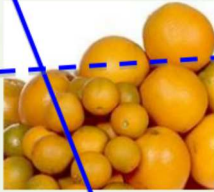


- ✓ No bactericide  
molecule so far.

Vector transmission  
capacity



Host susceptibility



sweet orange

Planting health young  
citrus plants

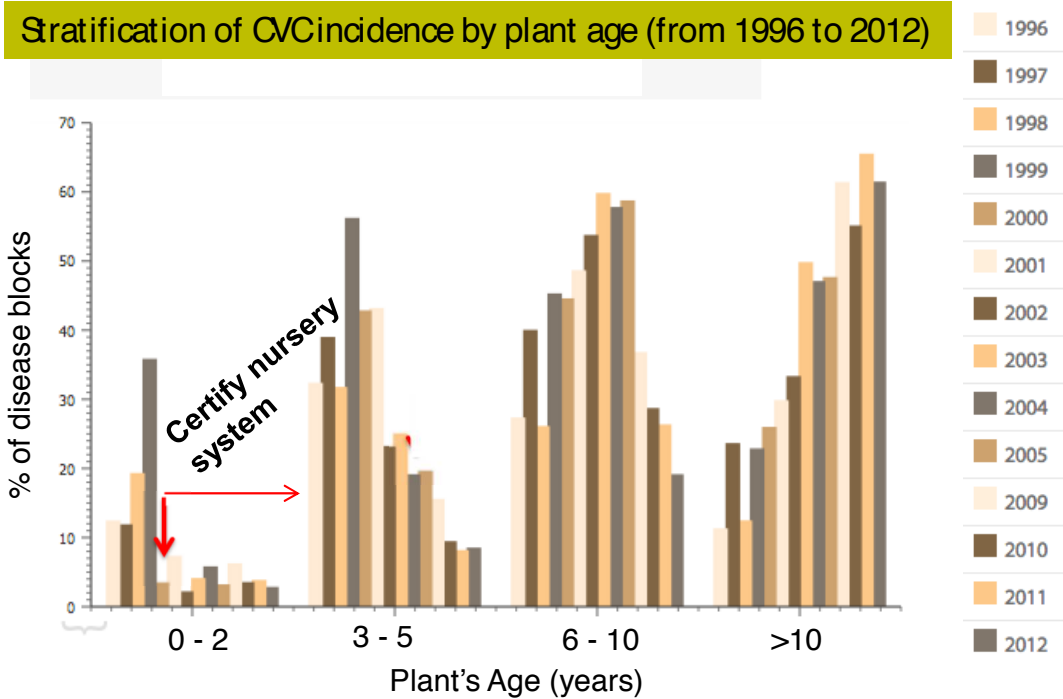
Reduction  
inoculum source

CVC Management – Health nursery plants

Planting health young citrus plants

✓ Early 2003: Well established system for production of citrus plants under vector-proof screen house.

Stratification of CVC incidence by plant age (from 1996 to 2012)



## CVC Management – Minimizing vector population

### Vector population control

- Inspection of sharpshooter population by yellow stick trap

## Chemical control

### Systemic and contact molecules

- Special attention to:
  - citrus blocks periphery and bordering forest edge
  - Spring and Summer seasons



### Systemic



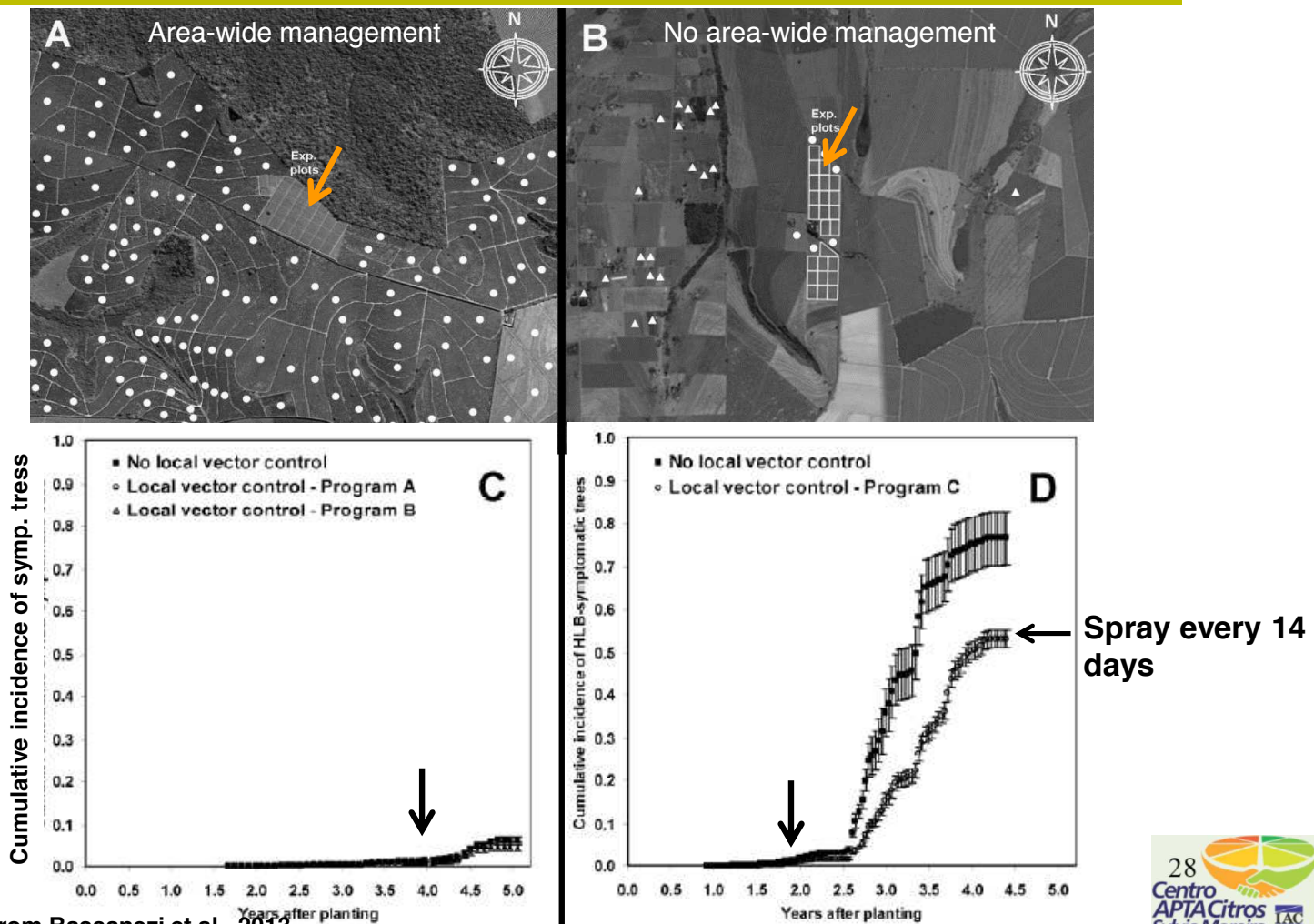
Young plants / soil humidity

### Contact



Older plants / dry seasons

Vector control, one example from HLB (greening) management program in Sao Paulo State



Adapted from Bassanezi et al., 2013



## Pruning of disease branches

## Not work

## Systemic distribution of XF in early infected plants

## 2 years old tree

### Initial symptoms level

**Leaves symp.**

## Leaves

## Sec. stems

## Main stem

## Trunk

## Analyzed samples

PCR +





## CVC Management - Reduction inoculum sources

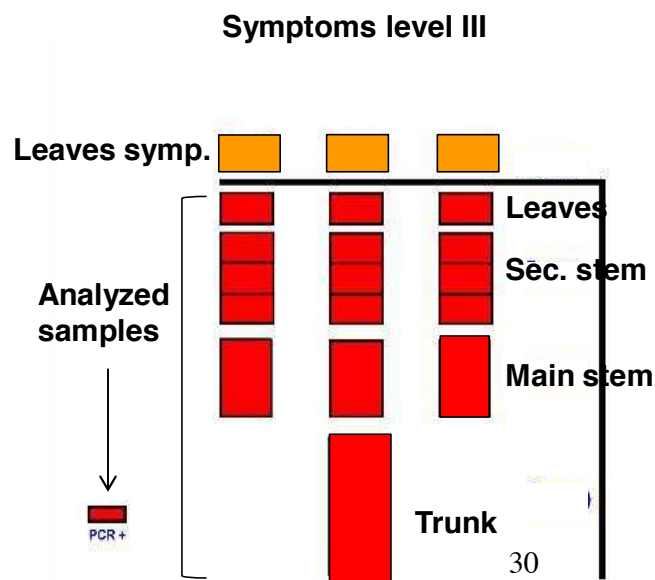
Pruning of disease branches

**Not work**

High infection level – no biologic death but strong reduction on productivity.



## Systemic distribution of XF in severely infected plants



CVC Management - Reduction inoculum sources

Pruning of disease branches

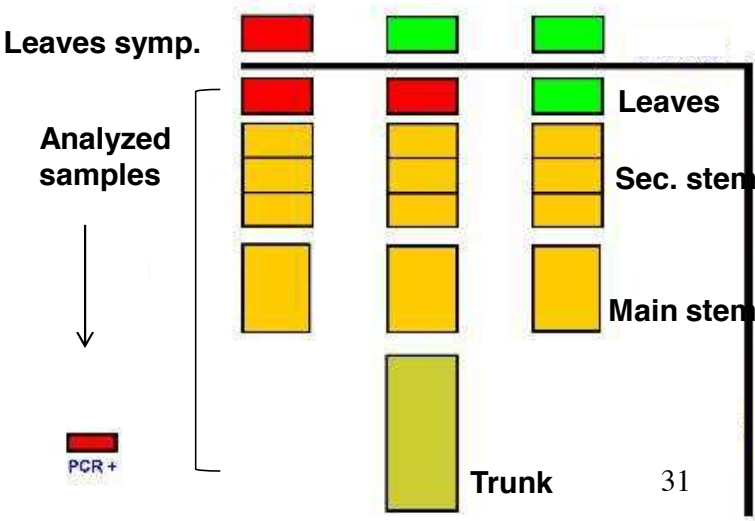
Work

Late and recent infections in 4 yr old plants or higher

Only in trees with initial leaves symptoms.



Systemic distribution of XF in plants with few symptoms



## Conclusion

- There is no a cake receipt or a silver bullet to solve bacteria-vector borne diseases.
- There is management strategies !
  - Each pathosytem, geographic region, seasons of year, and spots (blocks) required different intensity of actions within the management package.
- To know the biology and the management the vector population in the one of most important key to break the disease epidemiology.





**Thank you (obrigado)  
for your attention and  
time.**

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**Iguazu Falls - Brazil**



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FOZ DO IGUAÇU,  
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**SUSTAINABLE CITRICULTURE:**  
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Remaining **699** Days

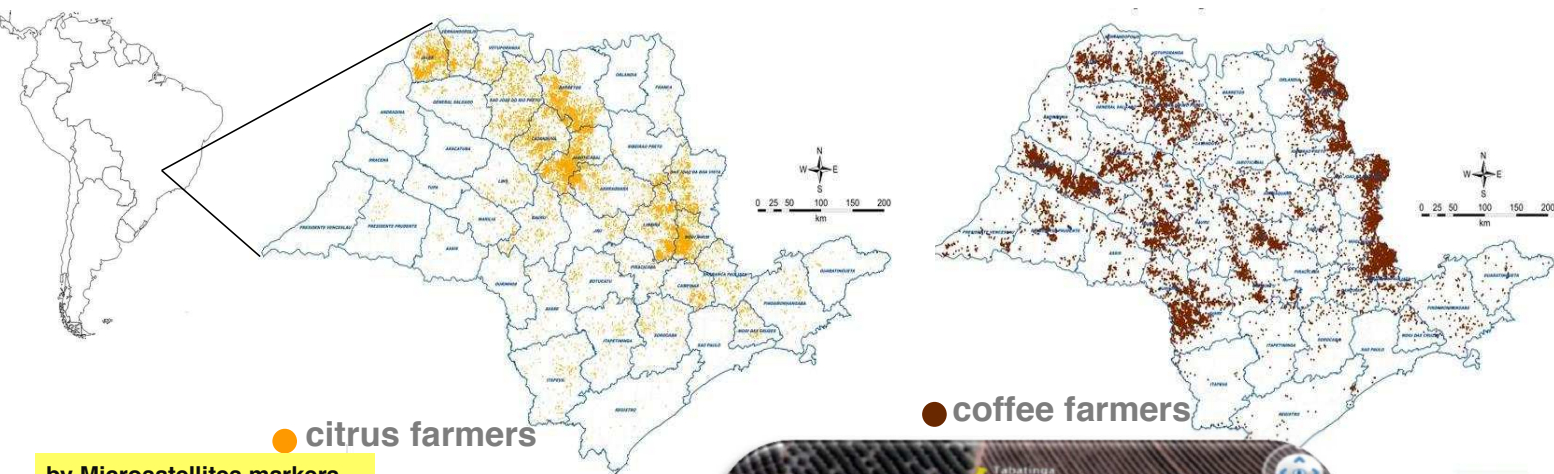






*X. fastidiosa subsp. pauca* { from sweet orange  
from coffee

## Geographic distribution of coffee and citrus farms in SPS, Brazil



by Microsatellites markers  
from 12 loci

