Experience in Americas, the endemic place for *Xylella spp.*

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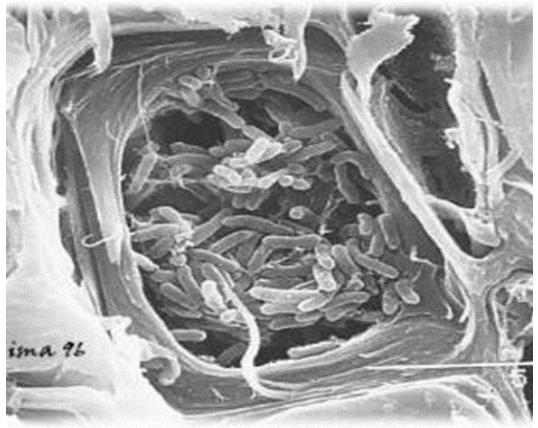
Xylella spp. – Taxonomy

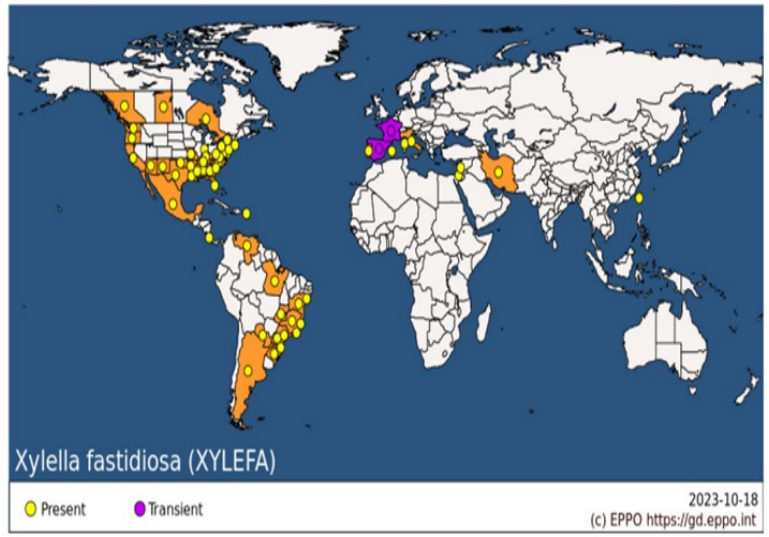
1 genus: *Xylella*
2 species: *X. taiwanensis* (Pear leaf scorch-only present in Taiwan)
X. fastidiosa (bacterial leaf scorch)

6 subspecies: subsp. *fastidiosa*
subsp. *multiplex*
subsp. *pauca*
subsp. *sandyi* * (*X. fastidiosa sensu largo*)
subsp. *morus* * (*X. fastidiosa sensu largo*)
subsp. *tashke* * (*X. fastidiosa sensu largo*)

* Genomic data doesn't support these subspecies.
* No original strains available for confirmation



SEM of Xf filled xylem of a coffee petiole © Jose Lima



✓ ***Xylella fastidiosa* is further characterised into Sequence Types**

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Visit to Brazil

Hosted by:

- Dr Helvécio Della Coletta-Filho,
  Instituto Agronômico de Campinas, Centro de Citricultura Sylvio Moreira, Cordeirópolis, **Sao Paulo**, Brazil.

  ✓ Learned a lot on Citrus Industry- CVC, HLB

- Prof Joao Lopes, Department of Entomology, University of Sao Paulo, ESALQ/USP, Piracicaba, Sao Paulo, Brazil

  ✓ Observed vectors inventory, vectors colonies, transmission assays, leafhoppers classification, surveillance methods

Citrus Industry in Brazil- Sweet oranges (*Citrus sinensis*)

- CVC is essentially a disease of sweet oranges. Sweet orange, the most important citrus cultivar in Brazil and is considered one of the most susceptible cultivars.

- Rangpur lime (*Citrus limonia Osbeck*), the most important citrus rootstock in Brazil, is resistant to CVC (Garcia et al., 2012).

- Xy doesn't infect lemons (*Citrus limon* (L.) Burm. f)

- No vertical transmission of the bacterium through sweet orange seeds originating from symptomatic fruits to sweet orange seedlings and no infection in lemon (*Citrus limon*) trees and seeds.

- Germplasm collection over 25 years old- Mother plants supply for breeding, budwoods- completely netted, sprayed and regular tested for seven diseases

- CVC- Citrus Variegated Chlorosis
- Nematode
- Citrus canker
- HLB- huanglongbing
- *Phytophthora spp*.
- Citrus tristeza virus
- Blackspot

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Xylella fastidiosa subspecies *pauca* (XFP) is dominant subspecies in Brazil

Plant Type	Disease caused by Xylella	*Xylella fastidiosa* subsp *pauca*
Citrus- Sweet orange	Citrus Variegated Chlorosis	subspecies pauca, **ST 11** and 13
Olives	Olive quick decline syndrome	subspecies pauca ST 16
Coffee	Coffee leaf scorch	subspecies pauca ST 16

- Sequence typing provides good insights into the phylogenetic position of the strains but is not clearly linked to host specificity, In citrus, two ST were determined, ST11 was common.

- Although there appears to be some degree of host specialization within the subspecies *pauca,* Cross-infection has been reported when artificially inoculated.

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Sequence Typing

- *X. fastidiosa* MLST by Scally et al 2005, Yuan et al 2010.
- 7 housekeeping genes *cys, gltT, holC, leuA, malF, nuoL, petC.*
- Different alleles for each gene- 156 alleles, 90 known STs (www.pubmlst.org).

cys, gltT, holC, leuA, malF, nuoL, petC.

1 1 1 1 4 1 1 = ST 2

- For example, *X. fastidiosa subspecies pauca*
  - Main hosts: Citrus present in South America has ST11 and ST13.
  - In 2013 a strain identified in olives in Italy, epidemic killed 21 million olive trees and massive loss to the industry. A new ST was determined as 53.

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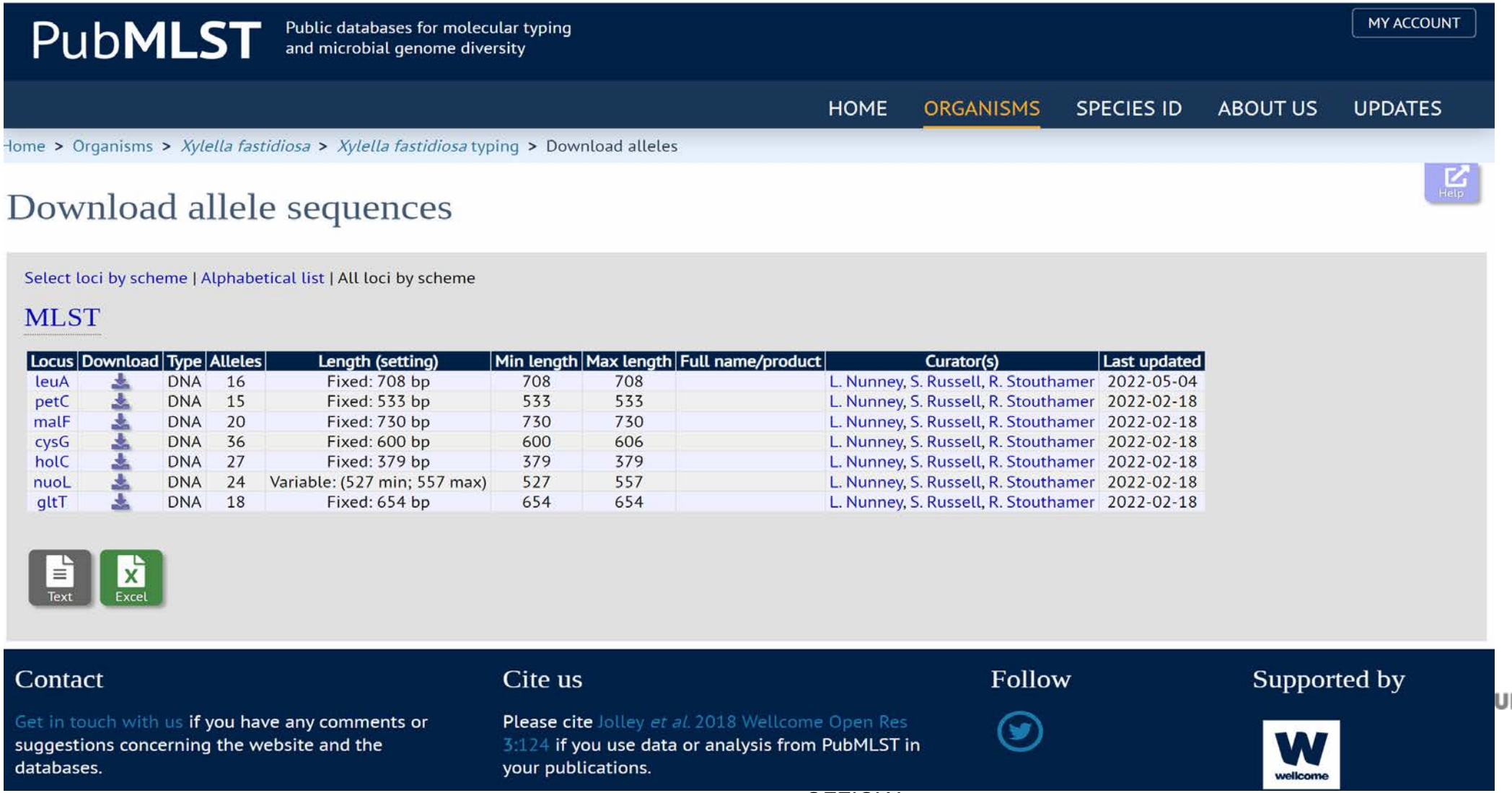
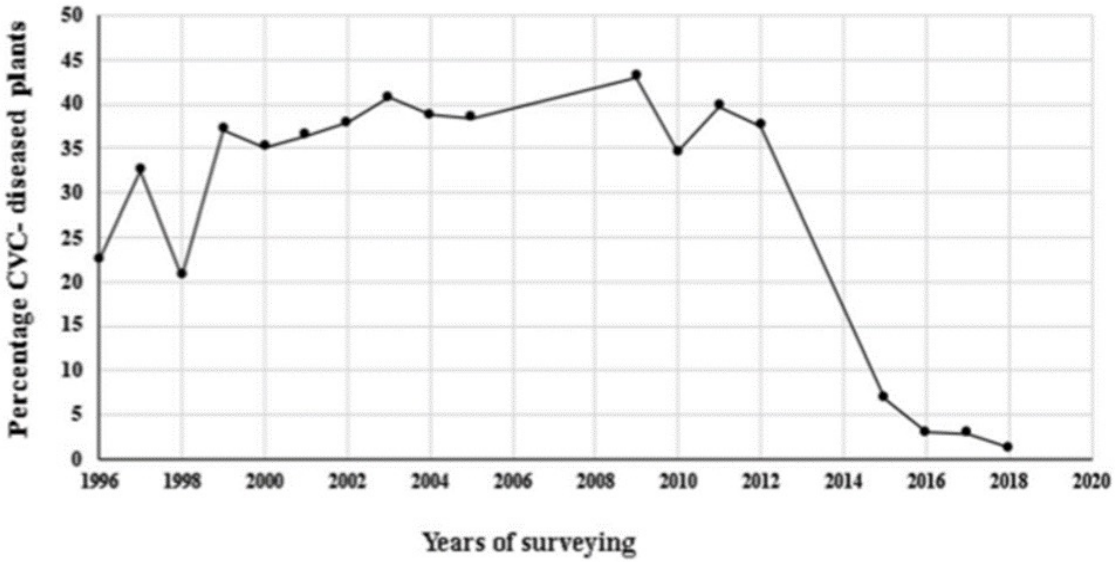
Xylella fastidiosa Sequence Types

https://pubmlst.org/organisms/xylella-fastidiosa



How Brazil have managed CVC

- At present only < 1% CVC is present in Brazil.
- A law was made in 2003 that all mother plants and propagation is done in net houses only. All budwoods were certified from the citrus centre.
- Rigorous testing of mother plants regularly.
- In 2004 HLB was spreading and lots of insecticides were used which were non-selective and controlled vectors of HLB and CVC.
- In 2008-2009 the price of citrus produce was down, and sugarcane was high, so farmers removed old citrus plants to grow sugarcane and therefore the inoculum was eradicated.
- Used resistant root stock that doesn't colonise bacteria.



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Symptoms of CVC in orange leaves

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Sampling

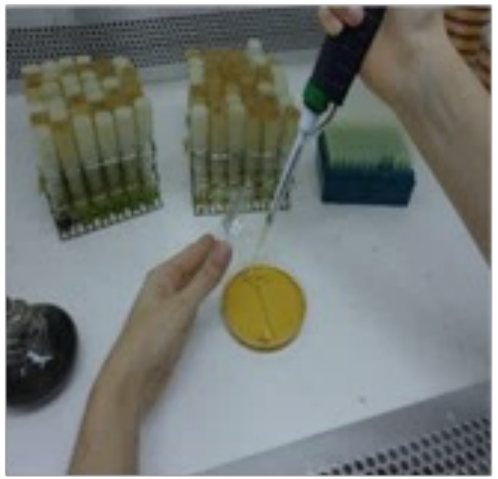
Petiole and mid ribs: Suitable for citrus plants for both bacteria and DNA isolation

Bacteria isolation:

1. Surface sterilized plant tissues with 70 % ethanol and bleach 1% (2min each) and 3 times wash with sterile water and dry in the hood.
2. Crush samples using PBS buffer in an extraction bags or in pestle and mortar.
3. Do x10 serial dilutions in PBS and plate 10^{-2}, 10^{-3}, and 10^{-4}
4. Buffered charcoal-yeast extract medium **BCYE** is a preferred medium, PWG also works

Petiole removal

Asepsis and cut the petioles



Plating of dilutions

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Olives

Early Olive Quick Decline Syndrome in Glasshouses

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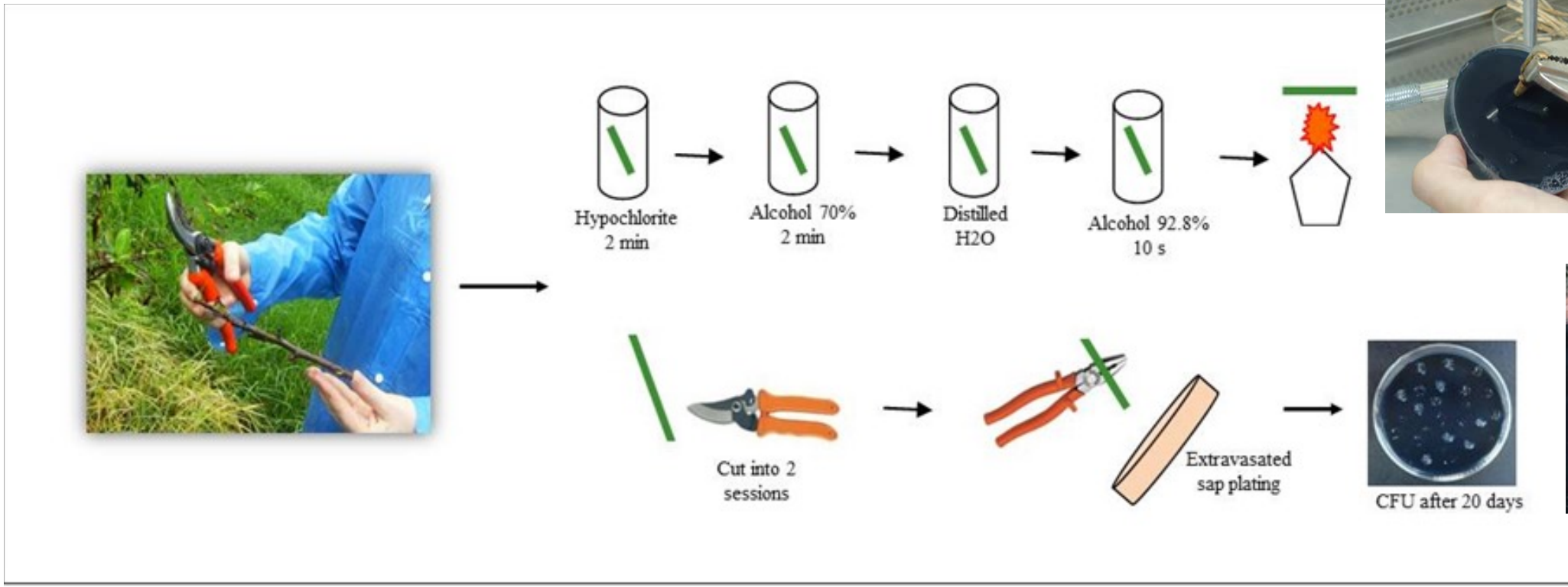
Sap methodology

Stems: suitable for Olive sampling for bacteria isolation



The first colony should appear after the 6th to 10th days BCYE medium. Stems are also used for DNA extraction.

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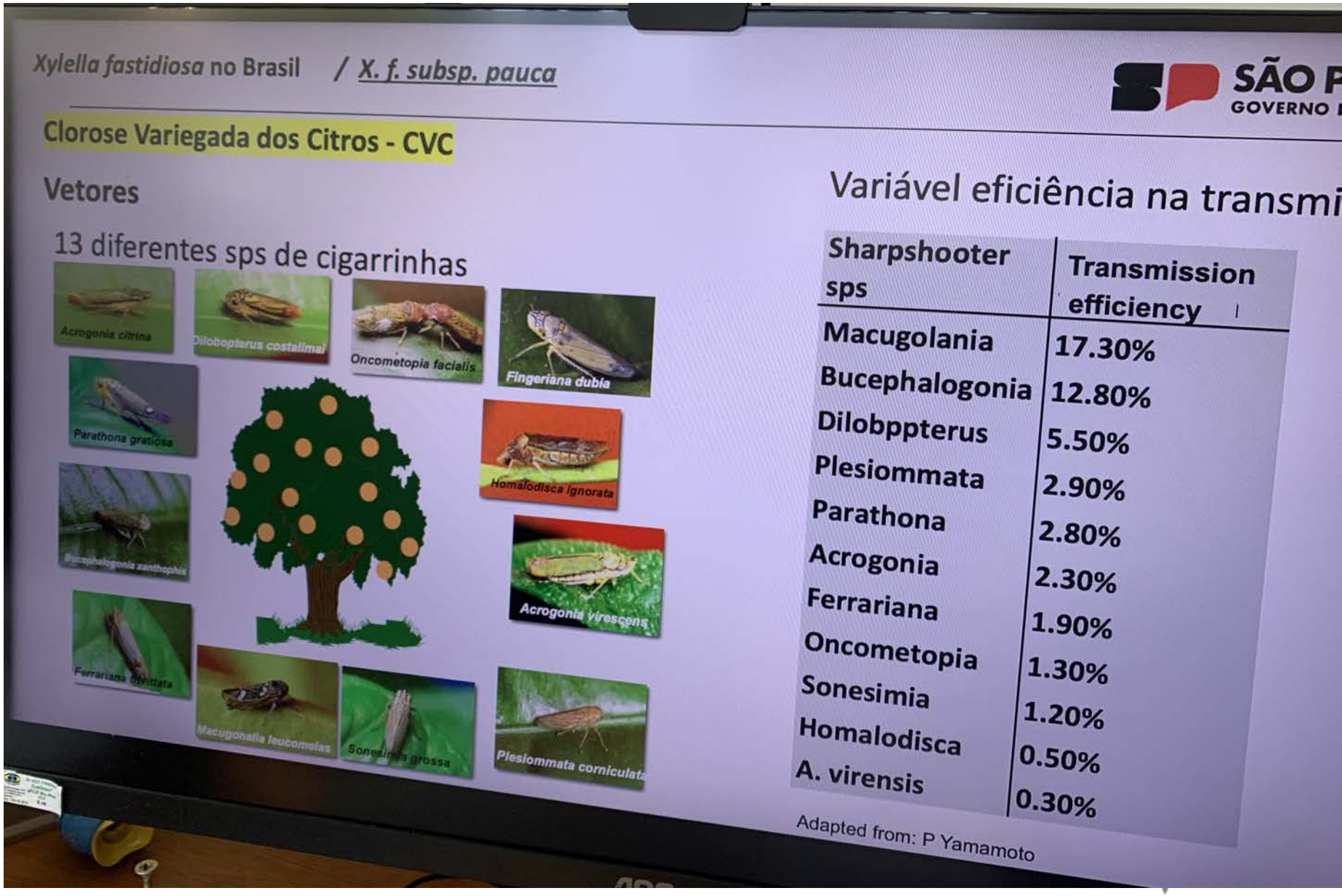
Vectors of *Xylella* in Brazil

13 different leaf-hoppers known to transmit *Xylella* in citrus



Vectors

Macugonalia leucomelas

family: ***Cicadellidae***

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Other learnings from Brazil – Huanglongbing (yellow dragon disease) HLB

Magnesium deficiency in citrus

HLB symptoms in citrus

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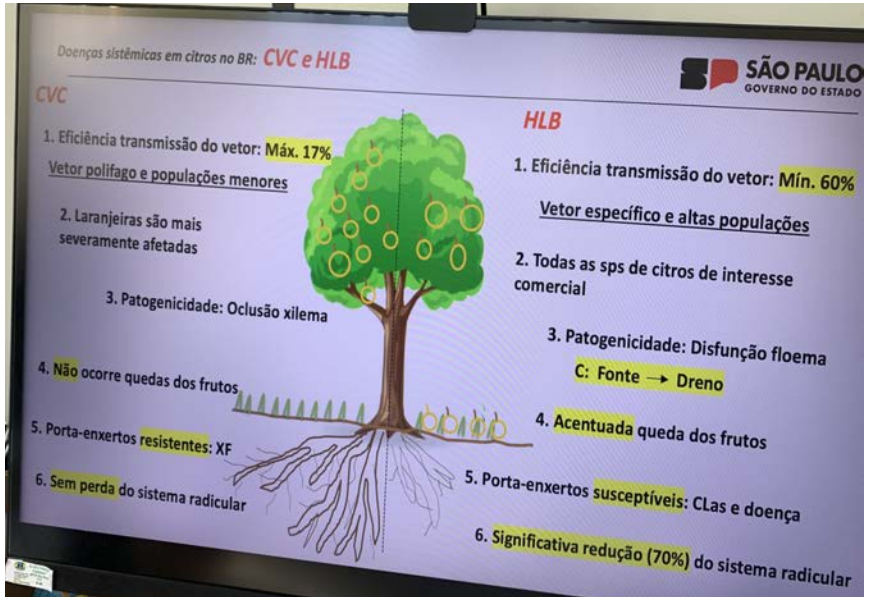
HLB infected orange fruit

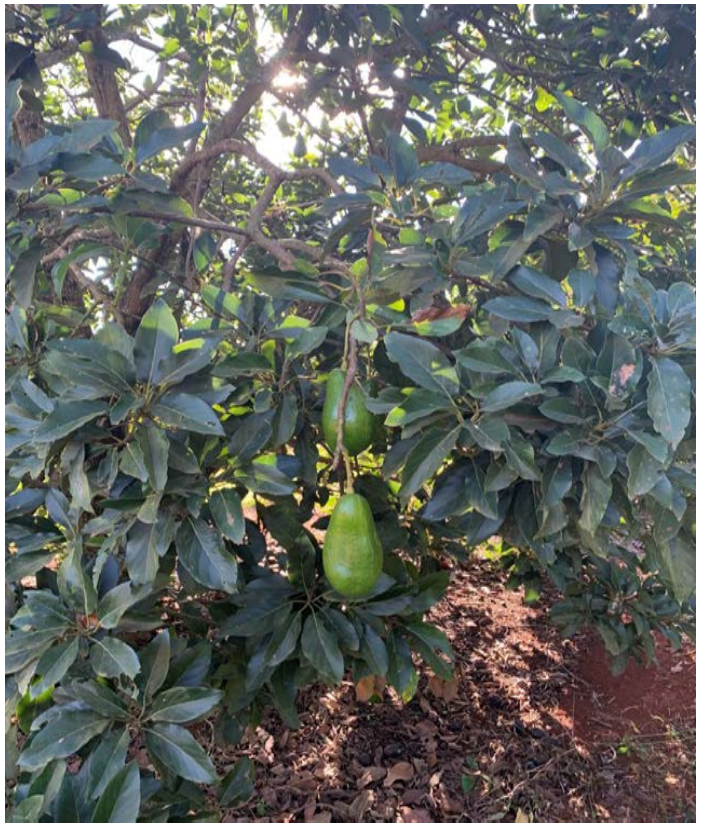
HLB caused by *Liberibacter americanus* symptoms in the sweet oranges

Differences between CVC and HLB

CVC	HLB
Small populations of many vectors	Very high population of the vector
Maximum efficiency of the vectors 17%	One vector with min efficiency is 60-100%
Orange trees are more severely infected	Infect all citrus species of commercial interest
Pathogenicity : Xylem occlusion	Significant fruit drop
No fruit drop	Pathogenicity due to phloem dysfunction
Resistant root stock- no colonisation of Xylella	Susceptible root stock
No loss of root system	70 % root stock is lost



Field trip to Citrus orchards



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California

USDA, Parlier, hosted by
Dr Lindsey Burbank

Elaine Backus – showed EPG, she runs online EPG workshop in small groups or even individually.

University of California, Berkely

Rodrigo Krugner – vibrations to stop glassywing sharpshooter mating in field settings

Scorching symptoms in Blueberry

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Almond leaf scorch infected with XFF

Inoculation of the grapevines



Symptoms of Pierce’s disease in red grape variety

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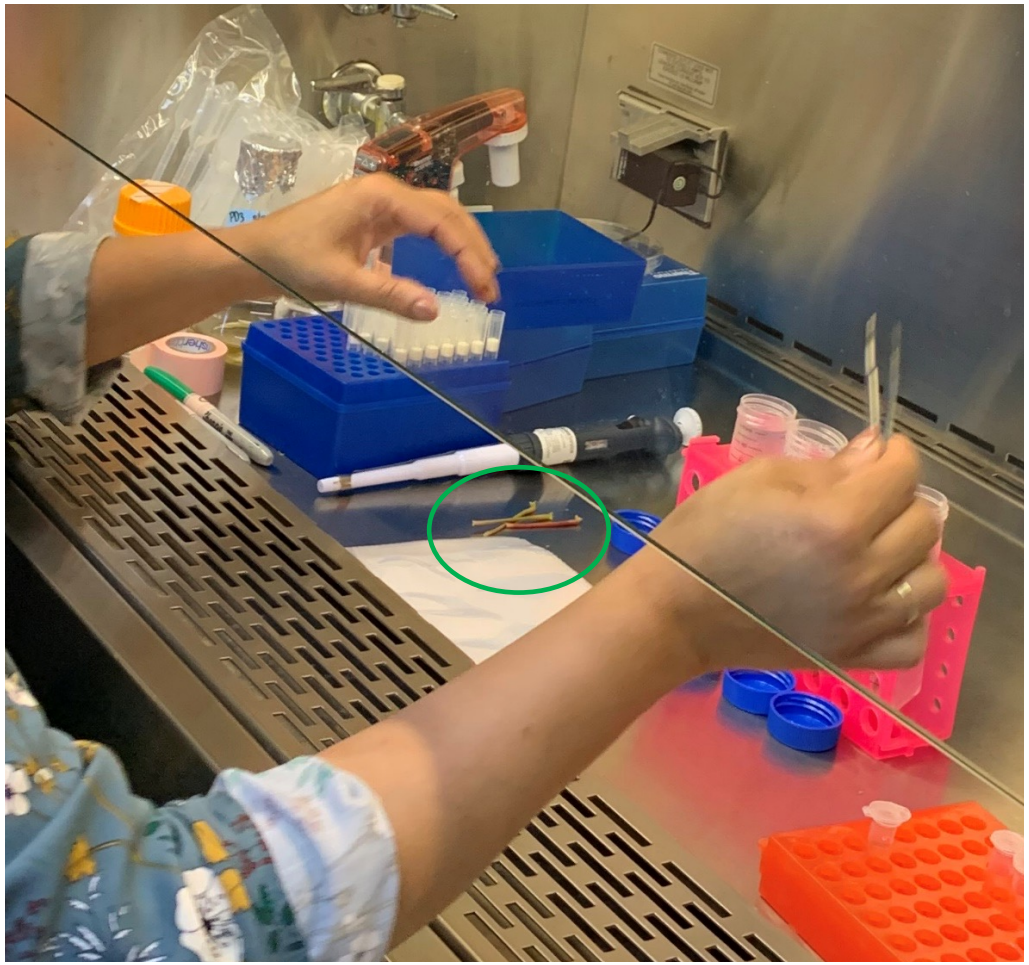
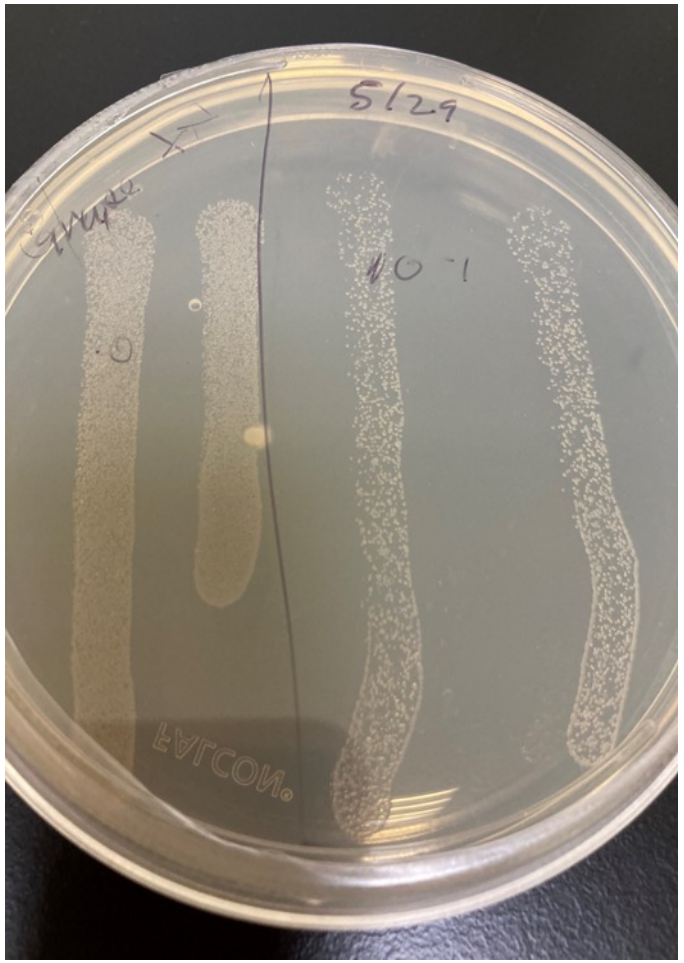
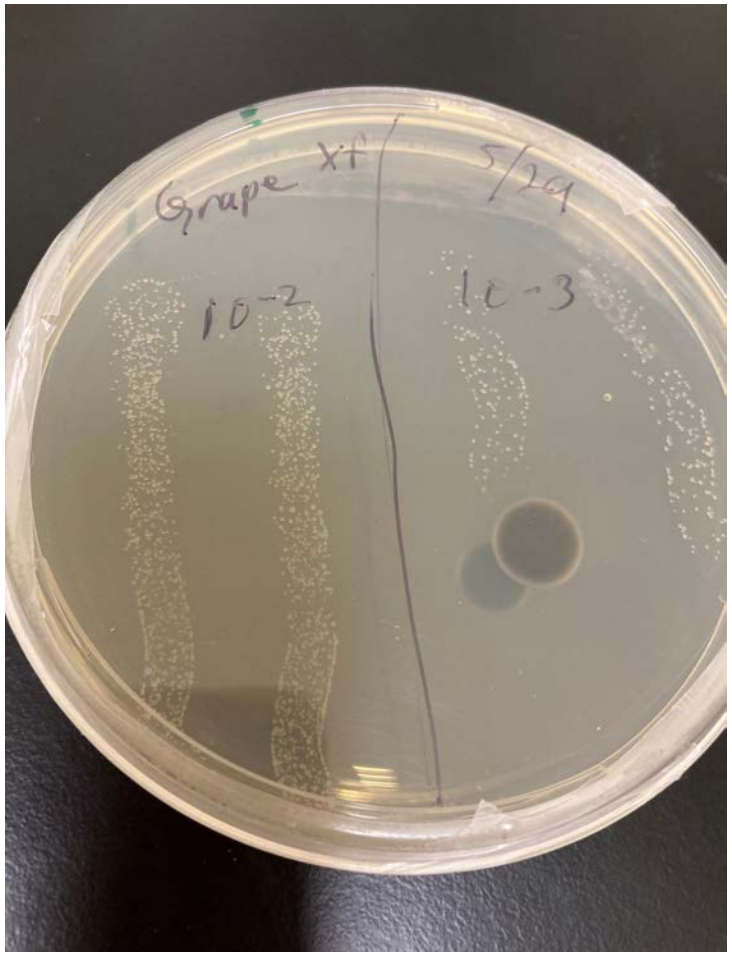
PD3 medium for XFF isolation from grapevines







Xylella ecology around Grapevines

University of California, Berkely

- Cooler temperatures helps in the plant recovery.

- *Xylella fastidiosa* in-vitro conditions does not multiply below 12-14 °C and slowly dies out below 4-5 °C (perhaps even lower than 8 °C

- Pierce Disease is not visible in lower temperatures, even in glasshouse, temp needs to be around 28-35 °C

- Xylella has two phases

1. Movement phase- hyper virulent where it is moving and degrading the pit membrane impaired the water uptake.
2. Biofilm phase – less active, blocking the vascular system, more chances of transmission by vector on feeding.

- XFM is been reported in trees around California. Surveying the trees on nature strips, for the presence of XFM with advices from local arborist.

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Acknowledgments

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