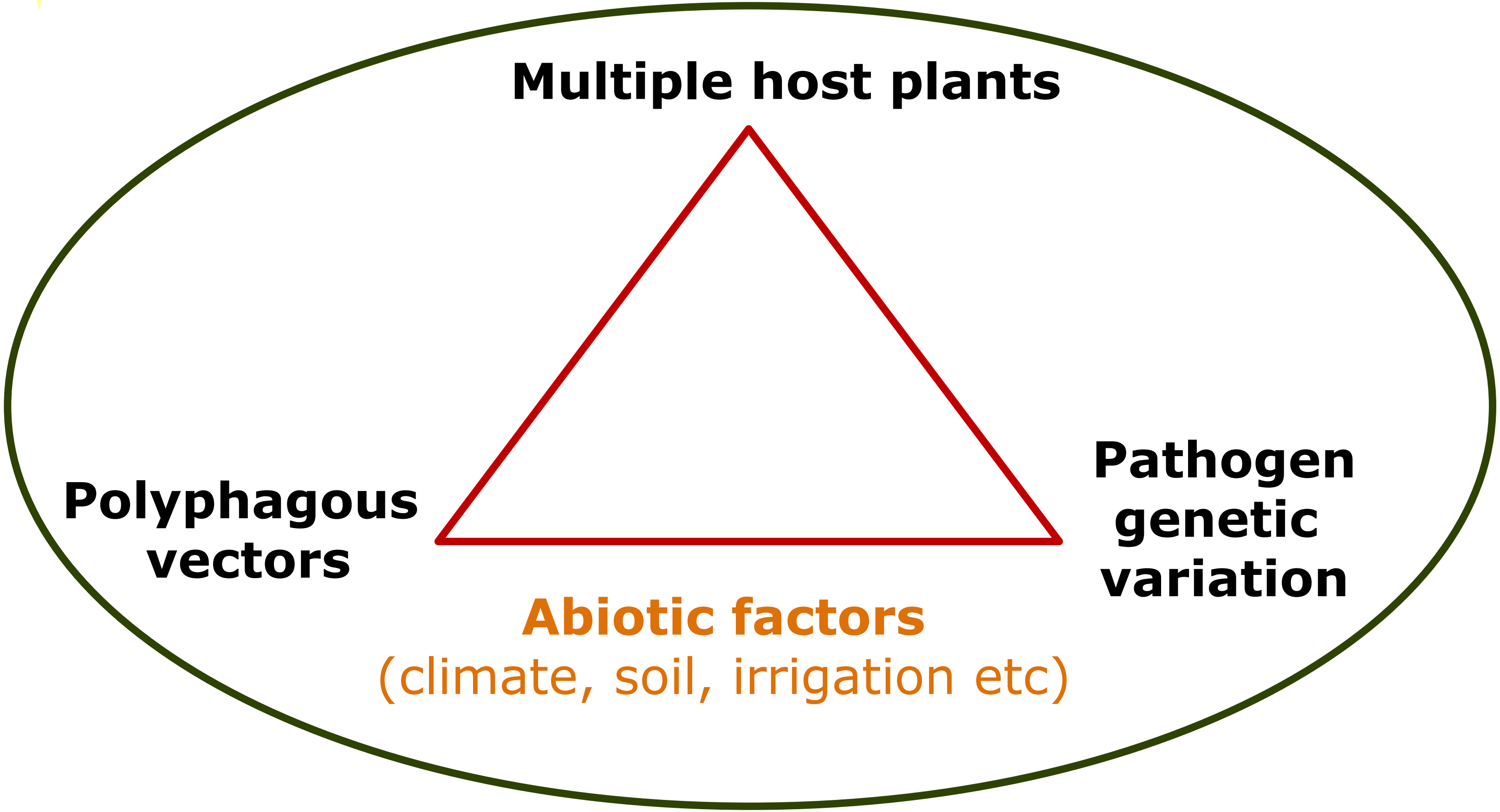
Diversity of xylem feeders and their role in epidemiology of diseases caused by *Xylella fastidiosa*

João R.S. Lopes
Dept. Entomology and Acarology
University of São Paulo/ESALQ, Brazil

Xf pathosystems – High complexity



Multiple vector species

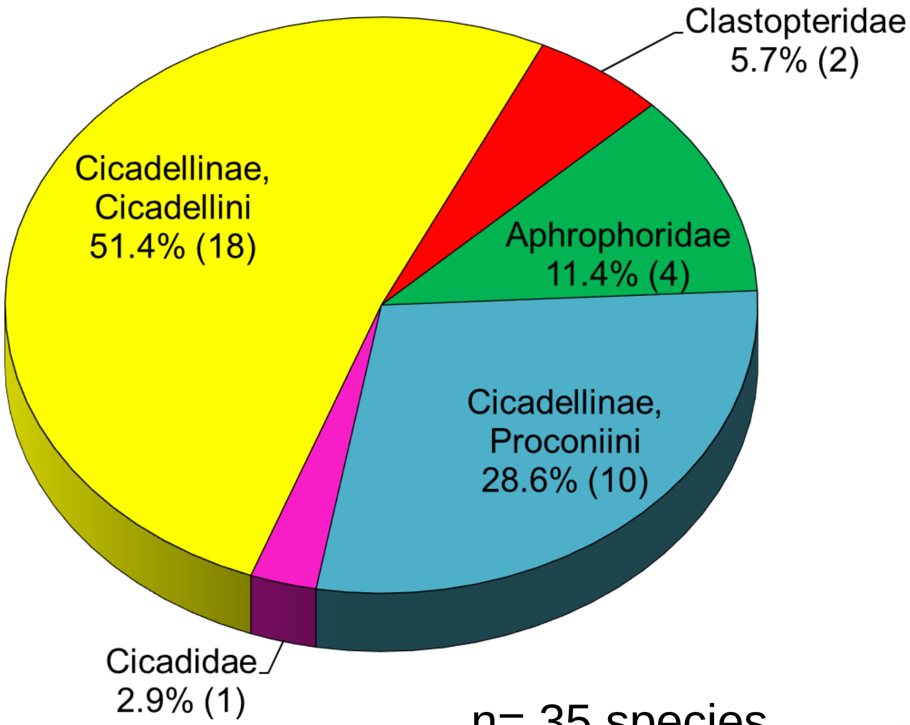
- What vector species play a major role in disease progress (“key vectors”)?

- What is the role of communities of sharsphooters and spittlebug species in disease ecology?

➡ *Determining what vector species and inoculum sources are relevant for pathogen spread is basic to establish disease management strategies.*

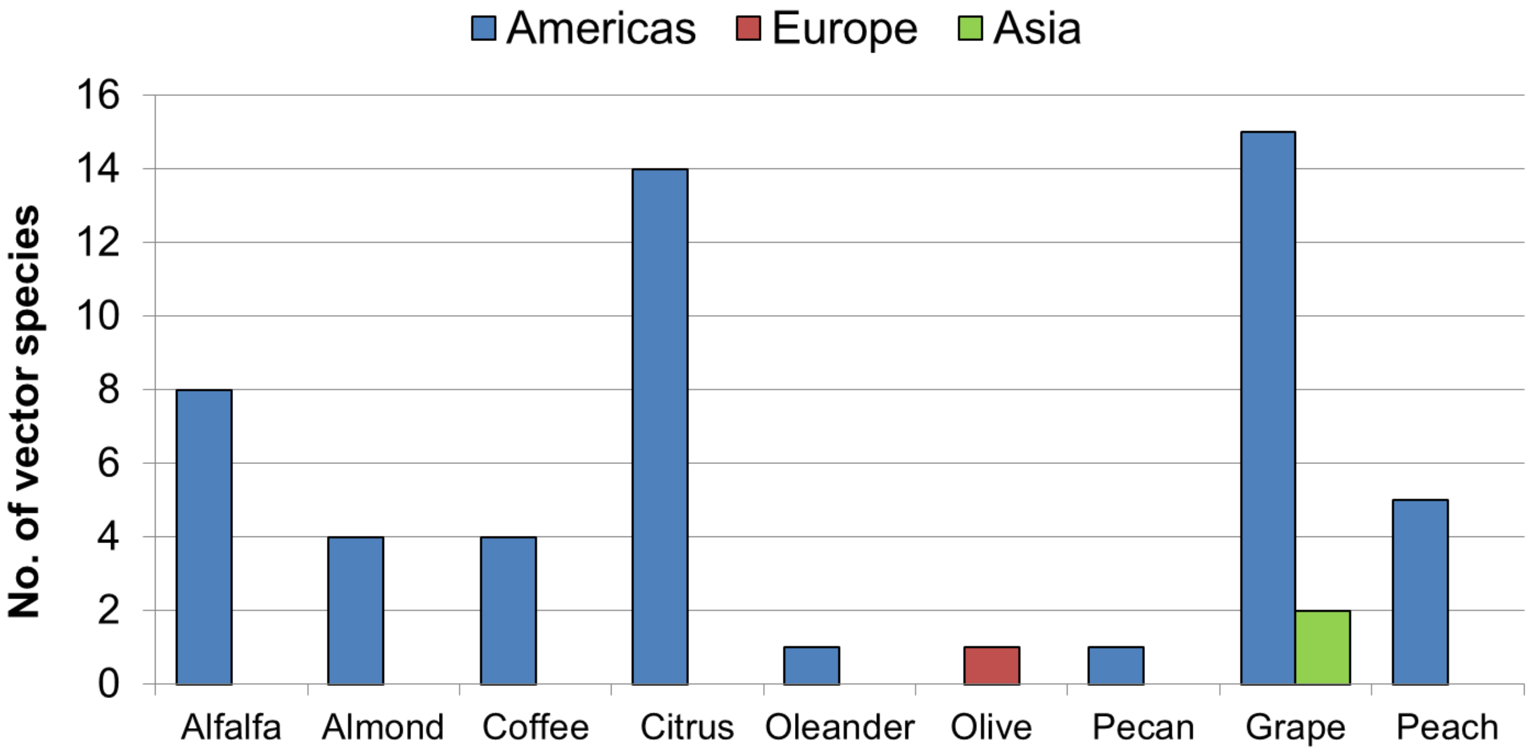
The vectors (xylem-sap feeders)

- **Sharpshooters:** **Cicadellidae, Cicadellinae**
- **Spittlebugs:** **Aphrophoridae Clastopteridae**
- **Cicadas**



n= 35 species

NUMBERS OF REPORTED VECTORS PER CROP



Vectors vs Potential Vectors

- Low vector specificity → many "potential vectors"
- Epidemiological role is not well known for most of the vector and potential vector species

Case study: Citrus Variegated Chlorosis (CVC) in Brazil

29 genera of xylem-feeding Auchenorrhyncha in citrus groves in São Paulo state, Brazil

Yamamoto & Gravena (2000); Giistolin et al. (2010)

Cicadellini

Bucephalogonia
Carneocephala
Ciminius
Diedrocephala
Dilobopterus
Erytrogonia
Ferrariana
Hortensia
Macugonalia
Oragua
Parathona
Plesiommata
Scopogonalia
Sibovia
Sonesimia
Syncharina

Proconiini

Acrogonia
Dechacona
Egidemia
Homalodisca
Molomea
Oncometopia
Pseudometopia
Teletusa
Tapajosa

Cercopoidea

Deois
Neosphenorhina
Mahanarva
Zulia

Transmission assays in citrus (Brazil)

Groups tested

- Cicadellinae (Cicadellini): 13
- Cicadellinae (Proconiini): 5
- Cercopidae: 1
- Gyponinae: 3
- Membracidae: 1
- Aethalionidae: 1

Xylem feeders

TOTAL: 24 species

13 species transmitted Xf to citrus (only sharpshooters)

Lopes et al. 1996; Roberto et al. 1996; Krügner et al. (2000); Yamamoto et al. (2002, 2007); Lopes & Krügner (2016)

Tribe Cicadellini (9)

Tribe Proconiini (4)

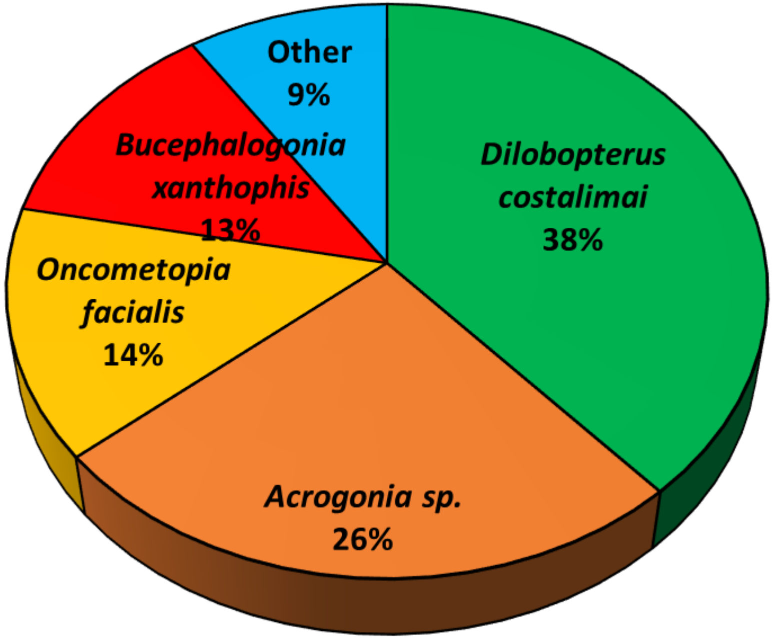
Most of them classified as predominant in citrus orchards by faunistic analyses (Giustolin et al. 2009)

Factors determining vector relevance

- **Predominance and activity**
- **Transmission efficiency**
- **Natural infectivity**
- **Host plants**
- **Inoculum sources (epidemiology)**

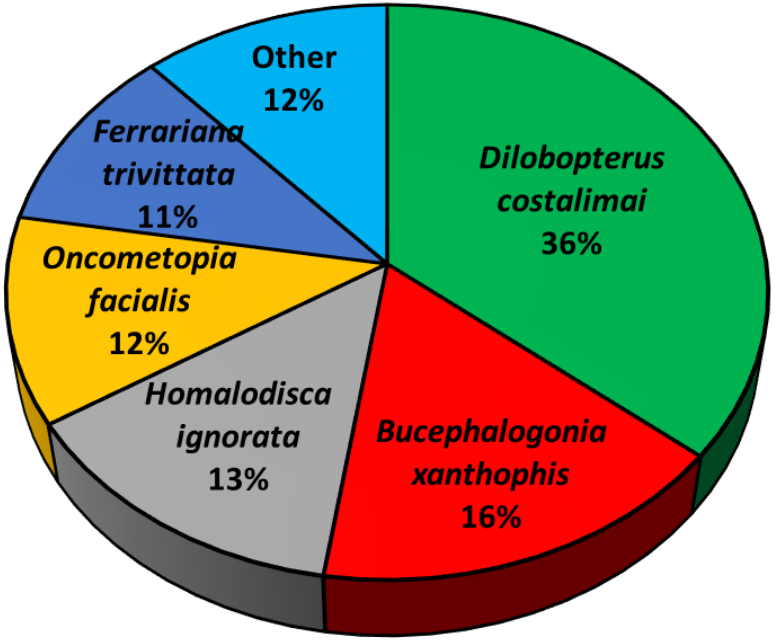
Activity of sharpshooter vectors in citrus orchards (Northern São Paulo State)

Yellow sticky traps (activity)



n = 649

Trap plants (visits on citrus)



n = 103

Pereira (2003)

Transmission efficiency to citrus

(Krugner et al. 2000; Yamamoto et al. 2002, Marucci et al. 2008)



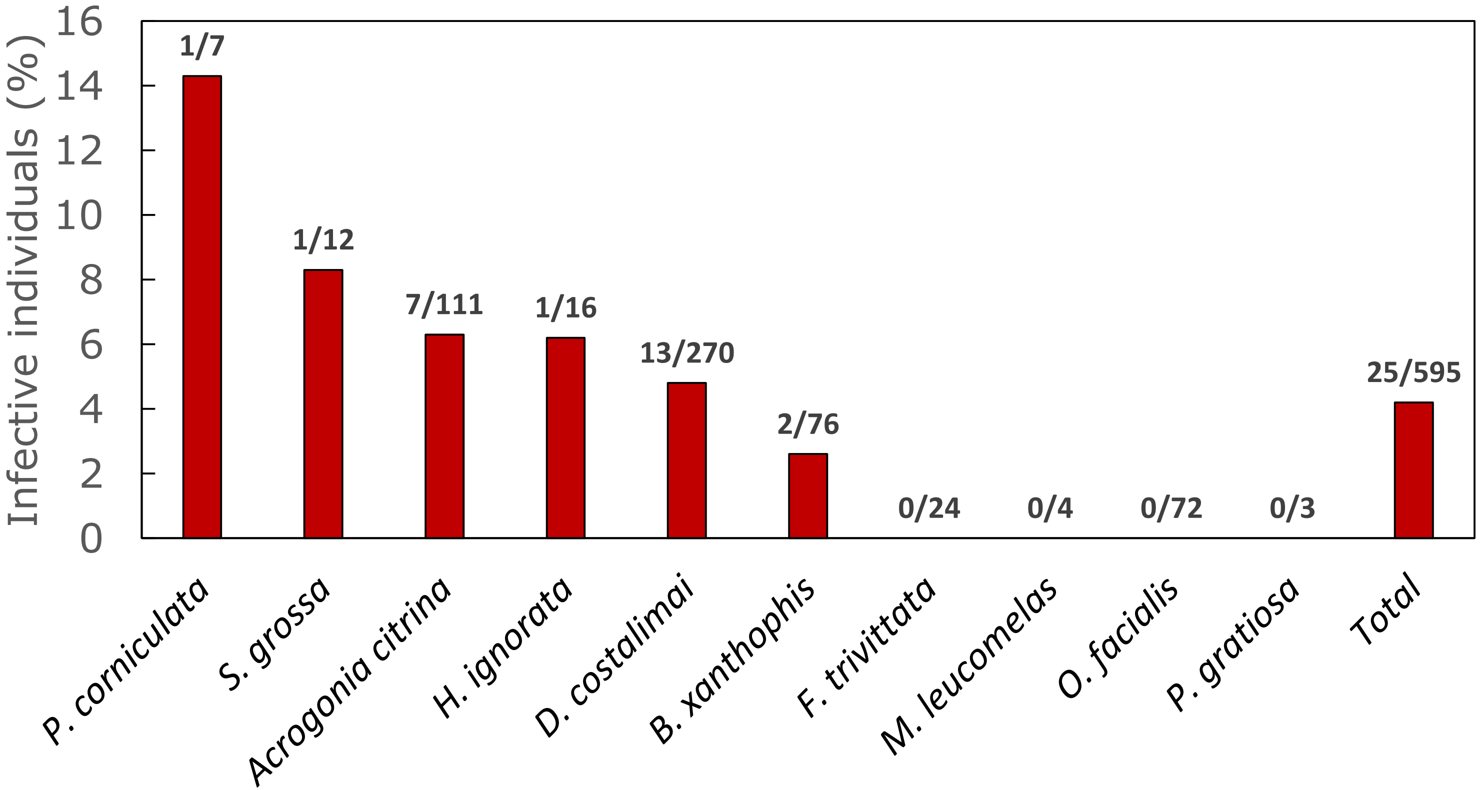


Macugonalia leucomelas	**17.3%**
Bucephalogonia xanthophis	**5.0-12.8%**
Dilobopterus costalimais	**5.5-13.3%**
Plesiommata corniculata	**2.9%**
Parathona gratiosa	**2.8%**
Acrogonia citrina	**2.3%**
Ferrariana trivittata	**1.9%**
Oncometopia facialis	**1.1-1.3%**
Sonesimia grossa	**1.2%**
Homalodisca ignorata	**0.5-30%**
Acrogonia virescens	**0.3%**



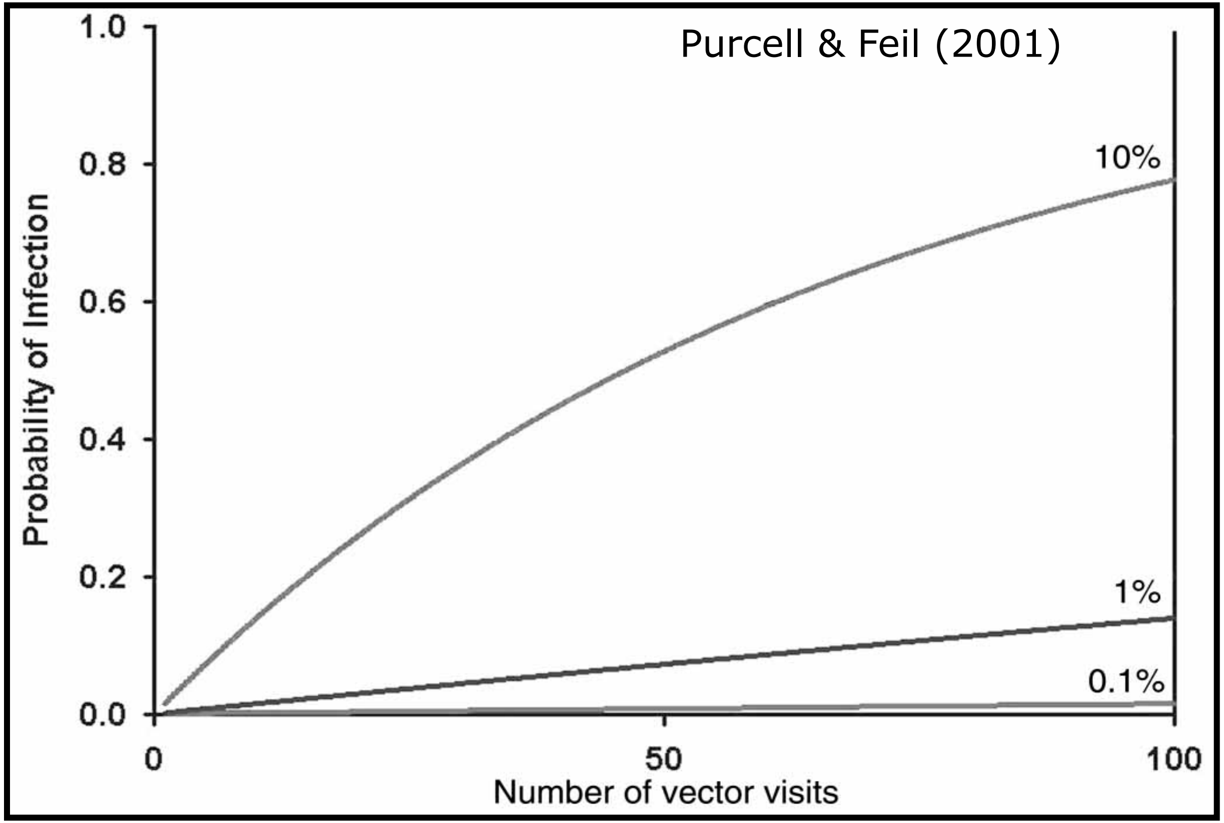



Natural infectivity of sharpshooters (ELISA-positive for Xf) in three citrus orchards (São Paulo State)



Pereira (2003)

Probability of infection as a function of the number of vector visits to plants and levels of infectivity, assuming a transmission efficiency of 15% per visit



Vector host plants and inoculum sources

- **Crops**
- **Herbaceous weeds**
- **Woody hosts (trees and shrubs)**

Distribution of prevalent sharpshooters in citrus groves

Paiva et al. (1996), Yamamoto & Gravena (2000), Giustolin et al. (2009)

- **Citrus canopy**: *Oncometopia facialis*, *Acrogonia citrina*, *Dilobopterus costalimai*, *Homalodisca ignorata*
- **Weeds and canopy**: *Bucephalogonia xanthopis*

Lay eggs and develop on citrus

- **Grass-feeders**: *Plesiommata corniculata*, *Ferrariana trivittata*, *Sonesimia grossa*, *Hortensia similis*

Abundant species on ground vegetation (rare on citrus trees)

Role of weeds as inoculum sources of *X.fastidiosa* in citrus orchards is unclear

Frequency of infection of weed mechanically inoculated with a CVC strain of *Xylella fastidiosa*

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

Family: Poaceae

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

Transmission assays to citrus using these hosts as source plants gave negative results (Lopes et al. 2003)

Some sharpshooters occur on woody habitats and host plants surrounding orchards

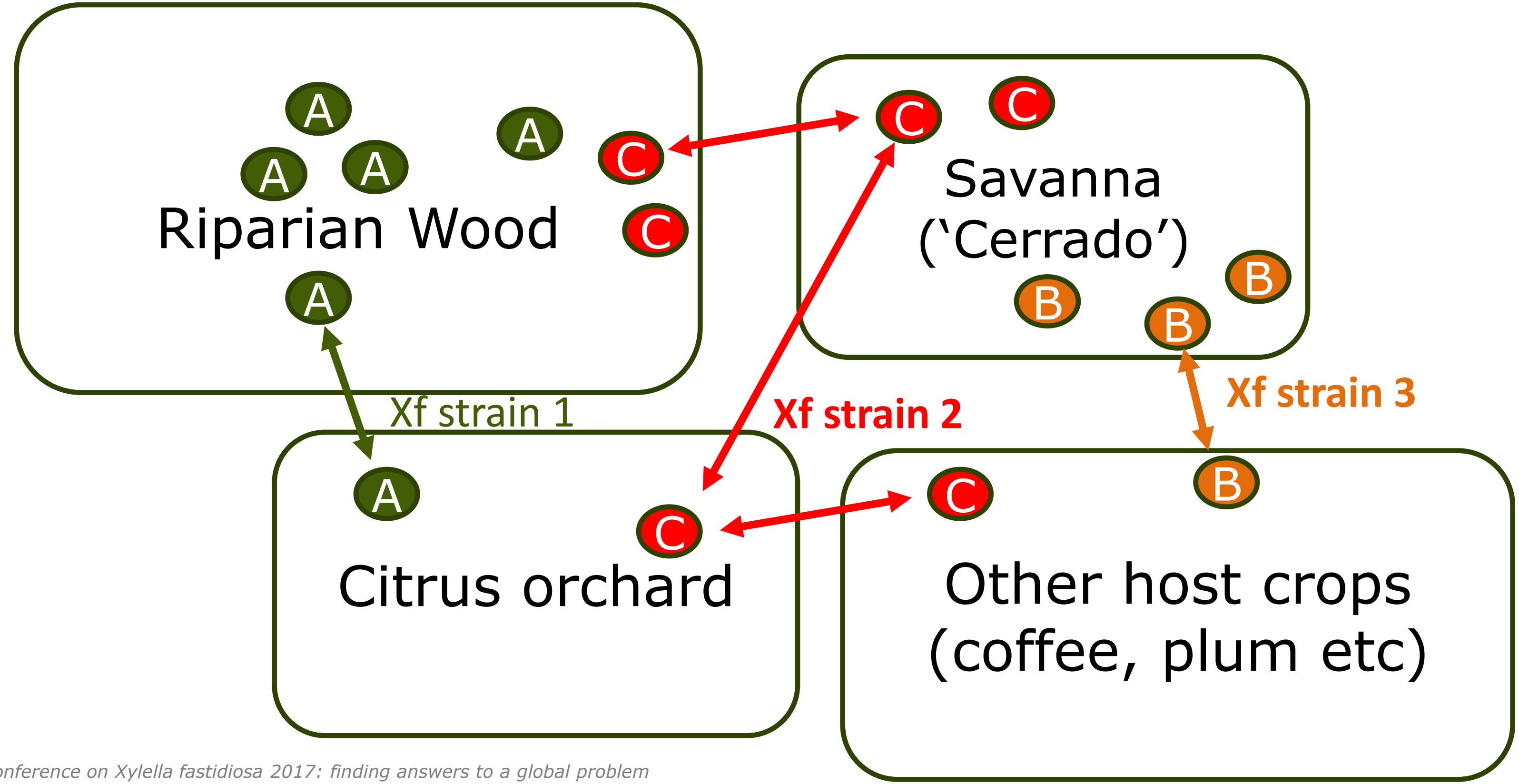
Around 40 host plants in 20 botanical families were identified as hosts of sharpshooter vectors in woody habitats in Sao Paulo State, Brazil (Lopes & Giustolin 2000)

Role of woody habitats and host plants on ecology of vectors and *X. fastidiosa*

- Refuge and breeding sites for several sharpshooters
- Source of vectors for orchard colonization after insecticide sprays
- Possible sources of inoculum and genetic diversity of the pathogen

FAPESP Proc. 01/05237-1

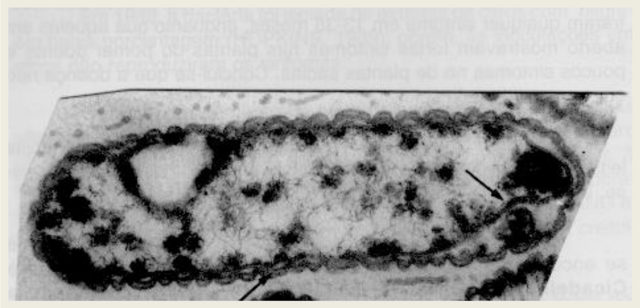
Polyphagous vectors (A B C) may carry *X.fastidiosa* strains among different host plants and habitats



Pierce´s disease in California North Coast

- Host plants of Xf and vector in riparian woods

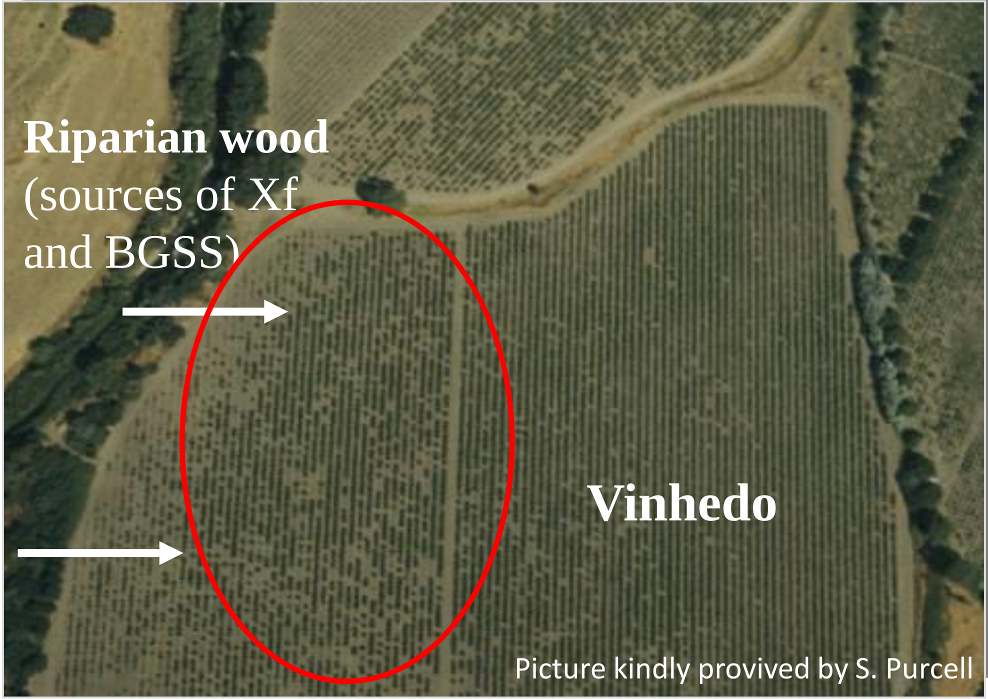
Graphocephala
Atropunctata
(Blue-green sharpshooter BGSS)



Pierce´s disease in California North Coast

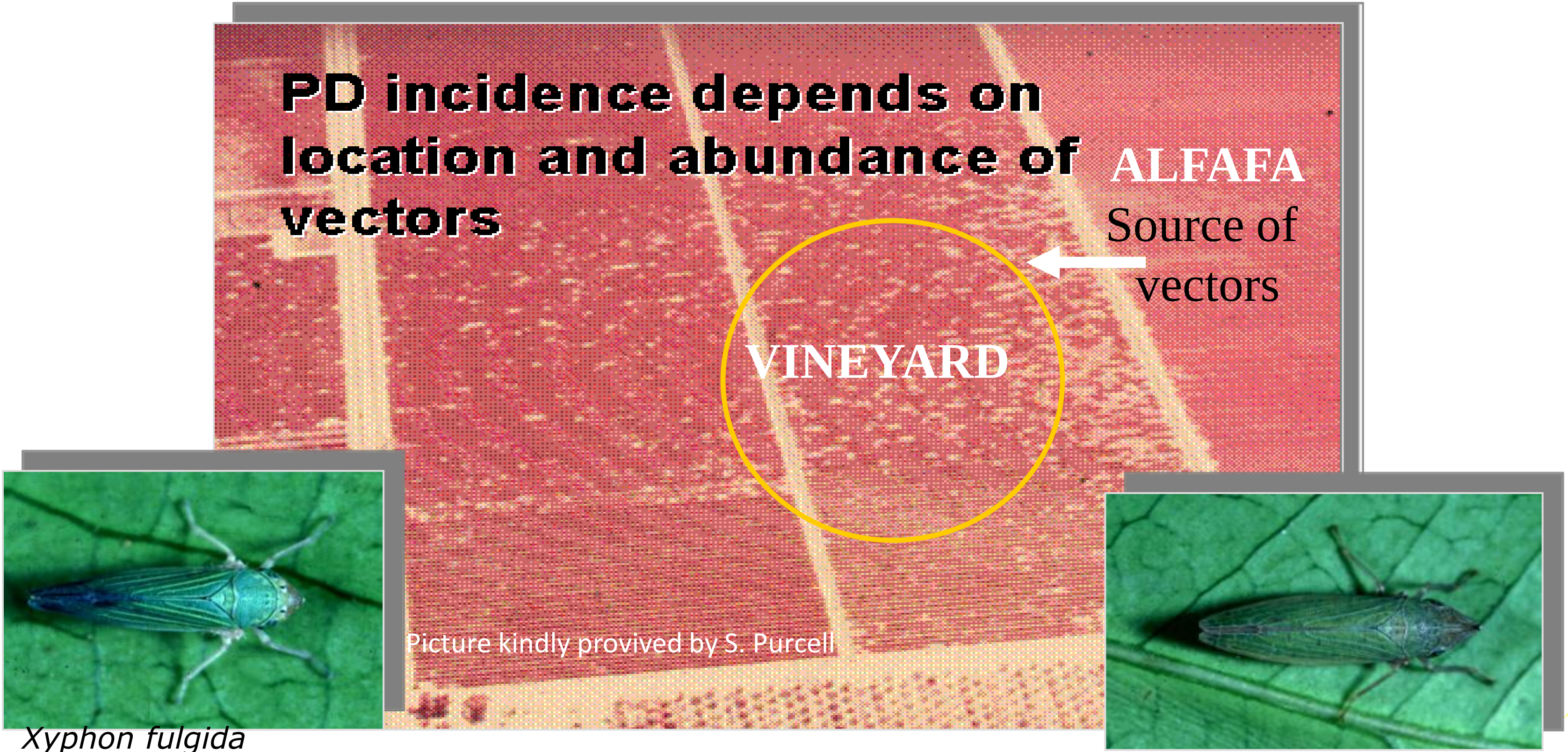
- Role of riparian wood as source of Xf and vector

Graphocephala atropunctata (BGSS)



Chronic infections in grape promoted by BGSS from inoculum sources in riparian woods (primary spread)

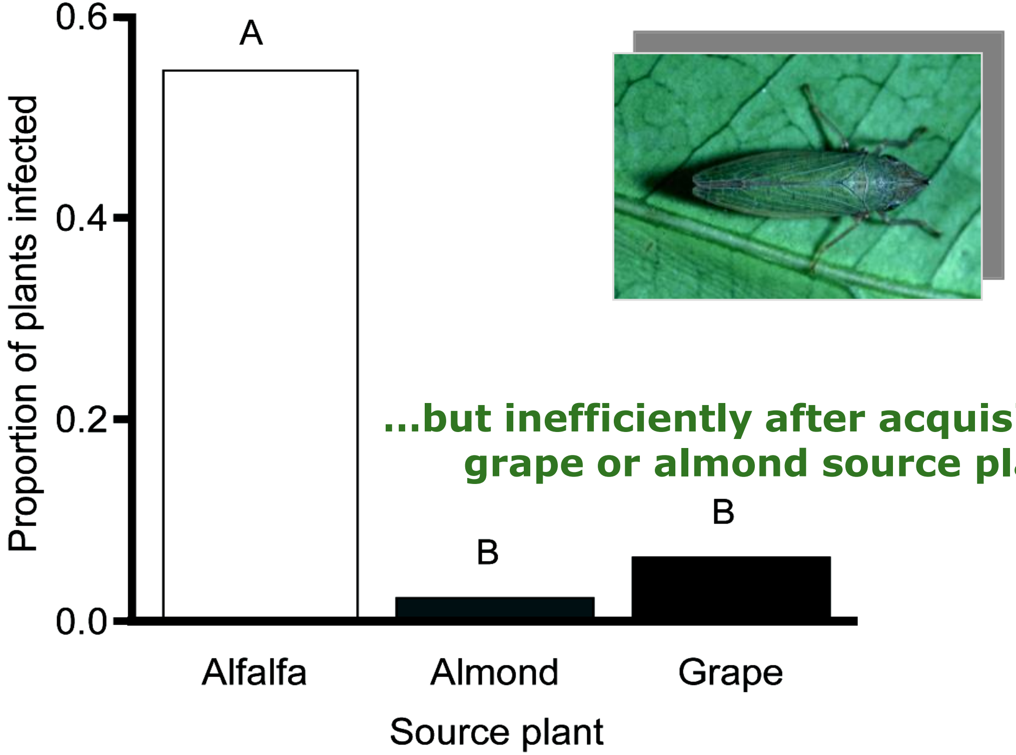
Primary spread of Pierce´s disease (PD) in California´s Central Valley



Xyphon fulgida
(read-headed sharpshooter)

Draeculacephala minerva
(Green sharpshooter – GSS)

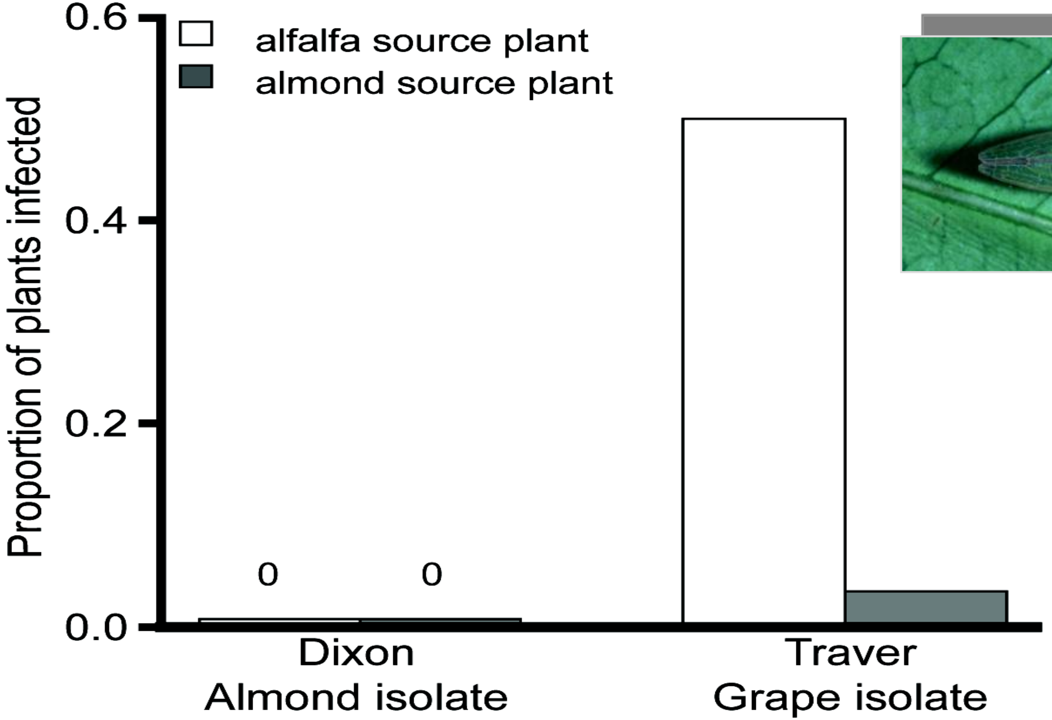
Draeculacephala minerva (GSS) efficiently transmits Xf to grapes after acquisition on alfalfa



…but inefficiently after acquisition on grape or almond source plants

Lopes et al. 2009

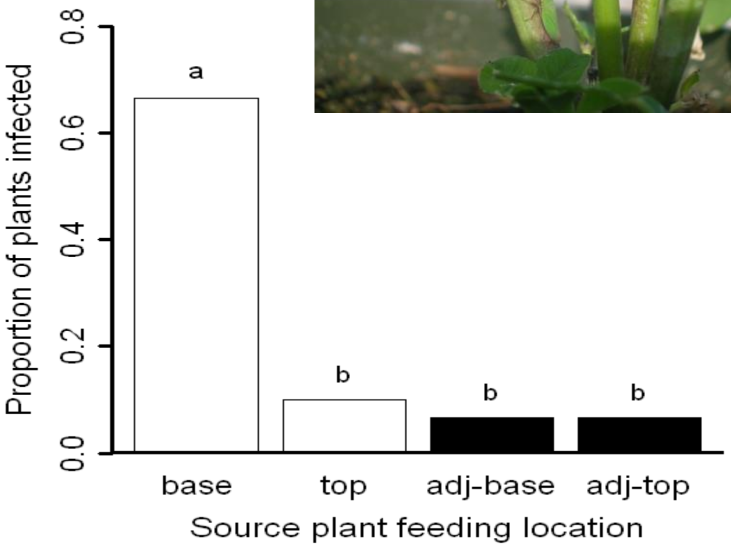
D. minerva (GSS) competence to transmit Xf from alfalfa depends on bacterium isolate/subspecies



X. f. subsp. mutiplex ***X. f. subsp.fastidiosa***

Lopes et al. 2009

D. minerva (GSS) prefers the basal part of the alfafa plant, where Xf population is higher and acquisition is more efficient



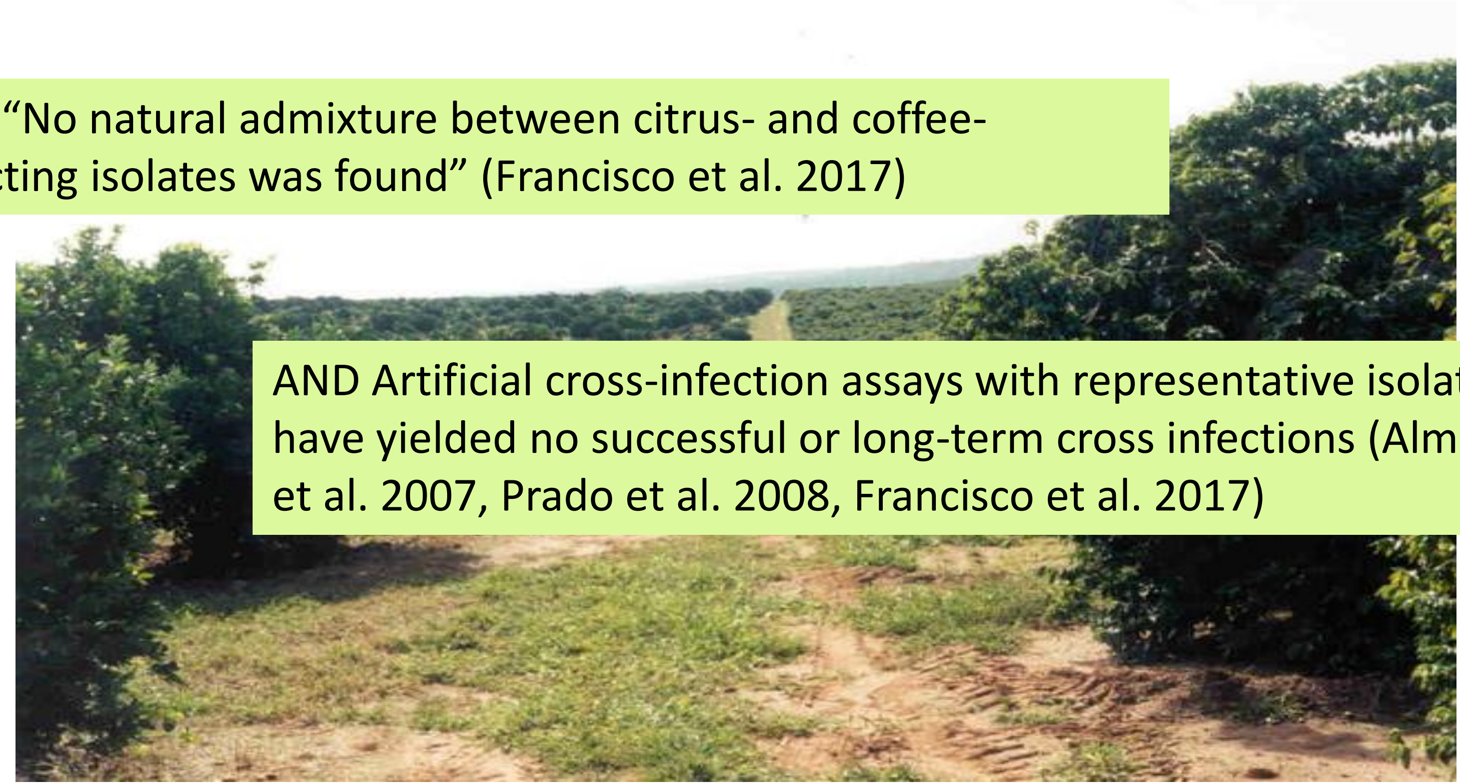
Daugherty et al. 2010

Inoculation and multiplication in the base of alfalfa plant is important for Xf survival, because alfalfa is harvested every 30-40 days

Therefore, vector feeding preferences and bacterial subspecies/strains can influence vector competence for pathogen spread from different inoculum sources

...and bacterial strains should be able to colonize both source and target plants

Citrus and coffee in Brazil: sympatric crops infected by closely-related Xf strains and several sharpshooter species in common



BUT "No natural admixture between citrus- and coffee-infecting isolates was found" (Francisco et al. 2017)

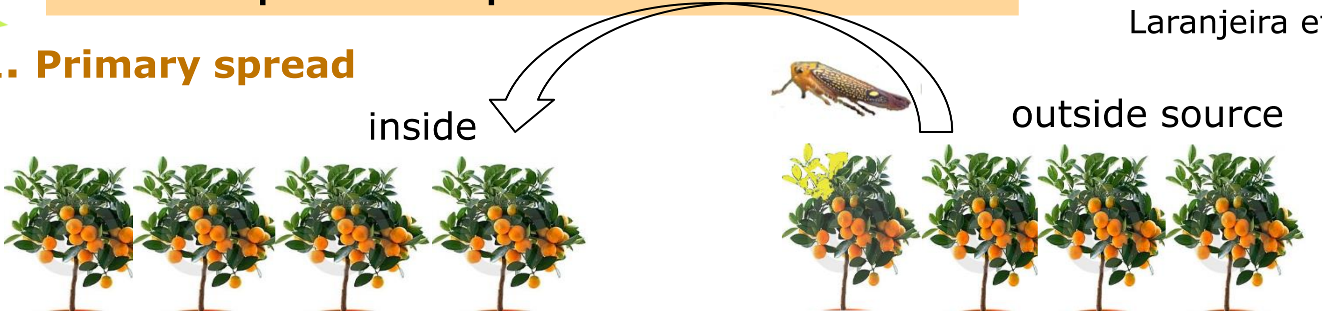
AND Artificial cross-infection assays with representative isolates have yielded no successful or long-term cross infections (Almeida et al. 2007, Prado et al. 2008, Francisco et al. 2017)

Epidemiology studies show that citrus is a major inoculum source for CVC

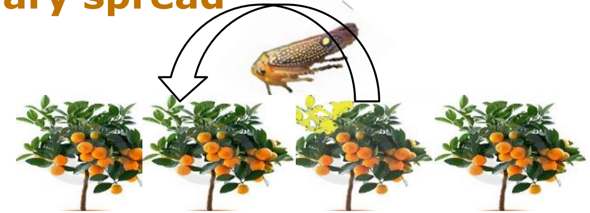
1ª and 2ª spread are important for CVC

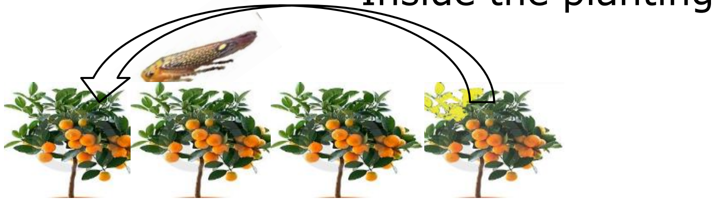
Gottwald et al. (1993);
Laranjeira et al. (1997, 1998, 2004)

1. Primary spread



2. Secondary spread





Key vectors in S. Paulo State

- (predominant species that colonize citrus trees)

Paiva et al. (1996); Lopes et al. (1999); Yamamoto & Gravena (2000); Giustolin et al. (2009)

New leaf scorching diseases in olives associated with *X. fastidiosa* in South America

Argentina

- **Haelterman, R.M. et al. (2015)** First presumptive diagnosis of *Xylella fastidiosa* causing olive scorch. Journal of Plant Pathology 97:393

Brazil

- **Coletta-Filho, H.D et al. (2016)** First report of olive leaf scorch in Brazil associated with *Xylella fastidiosa* subsp. *pauca.* Phytopathologia Mediterranea, temp 3-8

What are the vectors??

Symptomatic olive tree in Maria da Fé, Minas Gerais State, Brazil

WP5.1- Xylem-sap feeder communities in Southeastern Brazil (P11)

- Olive orchards over an altitudinal gradient - São Paulo (SP) and Minas Gerais (MG)

Sampling activities

Six localities in SP and MG:

Joyce Froza, Flavia Correr

- Wenceslau Braz/MG – 1780 m
- S. Bento do Sapucaí/SP – 1510 m
- Maria da Fé/MG – 1320 m (3 orchards)
- Consolação/MG – 1170 m
- Cabreúva/SP – 880 m
- Pilar do Sul/SP – 700 m

- **Yellow sticky cards**

- Sweep net (4 times/year)

WP5.1- Xylem-sap feeder communities in Southeastern Brazil (P11)

- Olive orchards over an altitudinal gradient - São Paulo (SP) and Minas Gerais (MG)

Partial Results (2 years of sampling)

Overall data (sticky traps-7 orchards):

Xylem feeders	No. spp.	No. Indiv.
Cicadellinae:	97 (17*)	11,748 (79%)
Cercopidae:	4 (2)	464 (3%)
Clastopteridae:	6 (1)	2,653 (18%)*

* Predominant species (highly abundant, highly frequent, constant and dominant)

Clastoptera sp. 1 was the only xylem feeder commonly observed on olive trees

Olive leaf scorch: many potential vectors, but no information on vector competence, host plant associations and epidemiology

Weeds and trees in the natural vegetation should be investigated as hosts of *Xf*

Spatial and temporal patterns of disease progress should be characterized

Final remarks and future directions

- Knowledge on vector competence, prevalence and host plant associations, well as on patterns of disease spread is critical for identification of key vector species.

- Vector colonization of affected crops is important for secondary spread within crops, but not essential for spread among different hosts and habitats

- Non-colonizing, but mobile vector species may still play important role in disease ecology.

- Ecology of *Xf* (inoculum sources) and vectors outside crops should be better understood.

Acknowledgements

Funding agencies: FAPESP (São Paulo Research Foundation) **Horizon2020** CNPq CAPES

Collaborators:

Alessandra de Souza; Helvécio Colleta-Filho (CCSM/IAC, Brazil)
Rodrigo Almeida and Sandy Purcell (UC-Berkeley, USA),
Rodney Cavichioli (UFFR, Brazil)
Wilson Azevedo-Filho (Univ. Caxias do Sul, Brazil)

Students/post-doc:

Current: Joyce Froza, Mariana Esteves, Flavia Correr
Former: R. Krugner, E. Pereira, T. Giustolin, R. Marucci, S. Prado, M. Miranda, R. Ringenberg, R. Marques, C. Müller, L. Graner, M. Esteves

Vector Lab at ESALQ/USP (http://www.lea.esalq.usp.br/labs.php)