PROGRESS AND PERSPECTIVES OF RESEARCHS ON HLB AND ITS VECTOR *Diaphorina citri*

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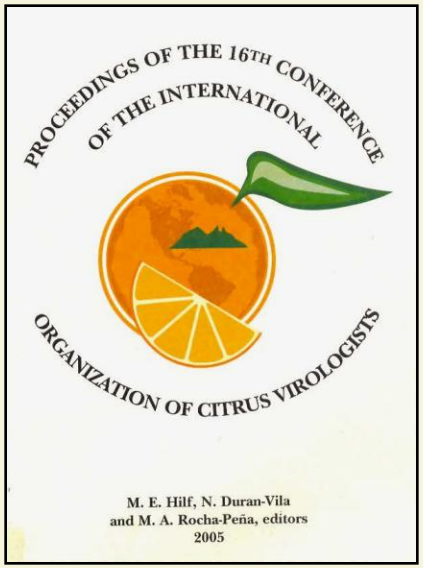
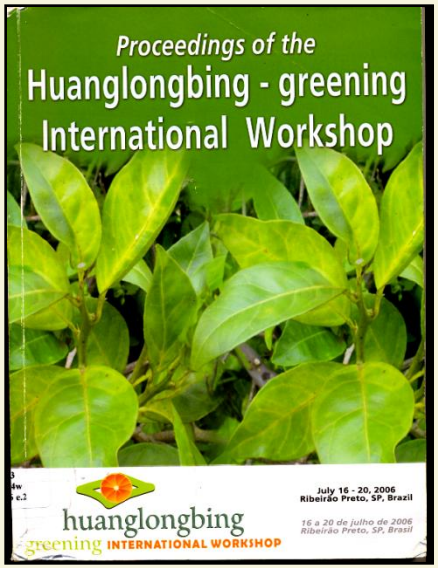
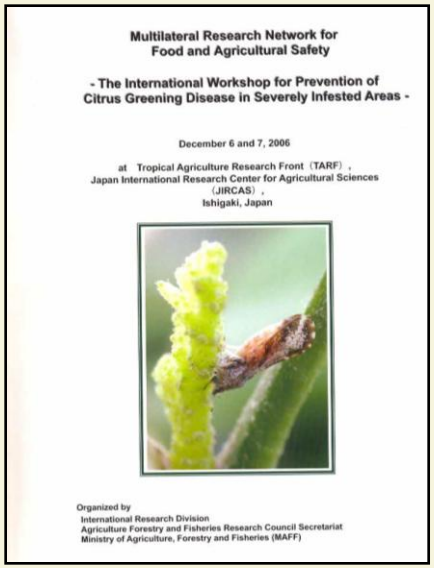
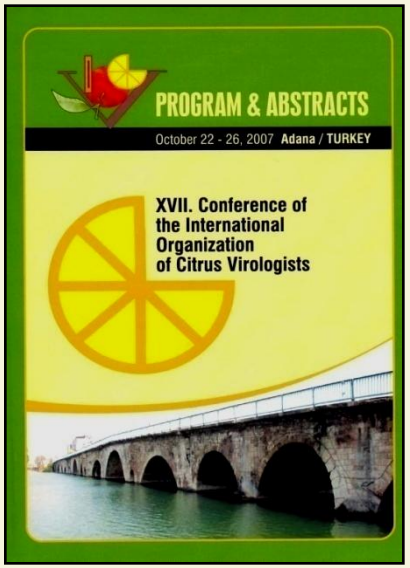
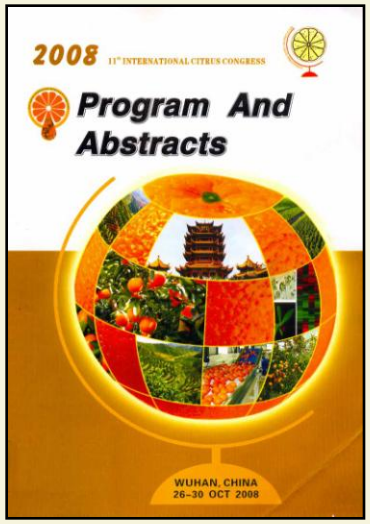
Source of information

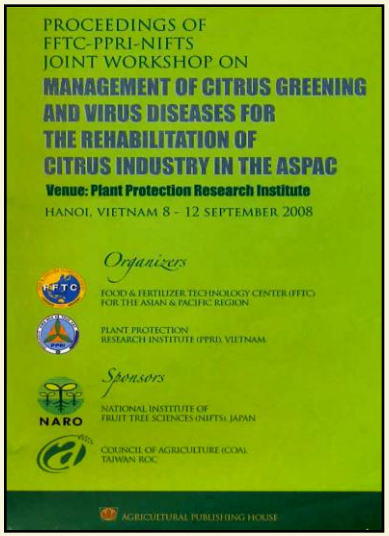


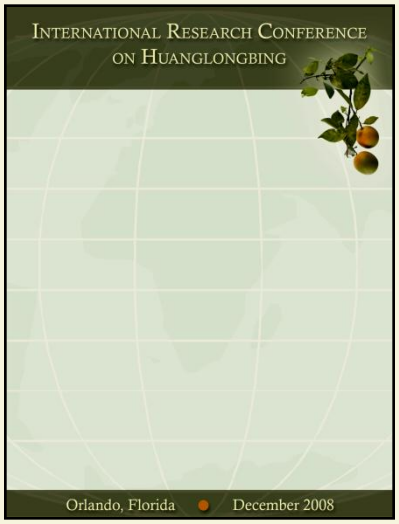












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Publications in periodical journals

Presentation topics

Introduction

- **HLB in the world**
- **HLB in Brazil**

Progress on HLB research

- **A new liberibacter in Brazil**
- **Phytoplasmas in Brazil and China**
- **Liberibacter detection and quantification**
- ***Ca.* L. americanus versus *Ca.* L. asiaticus**
- ***In planta* distribution**
- ***Murraya paniculata* as a host**

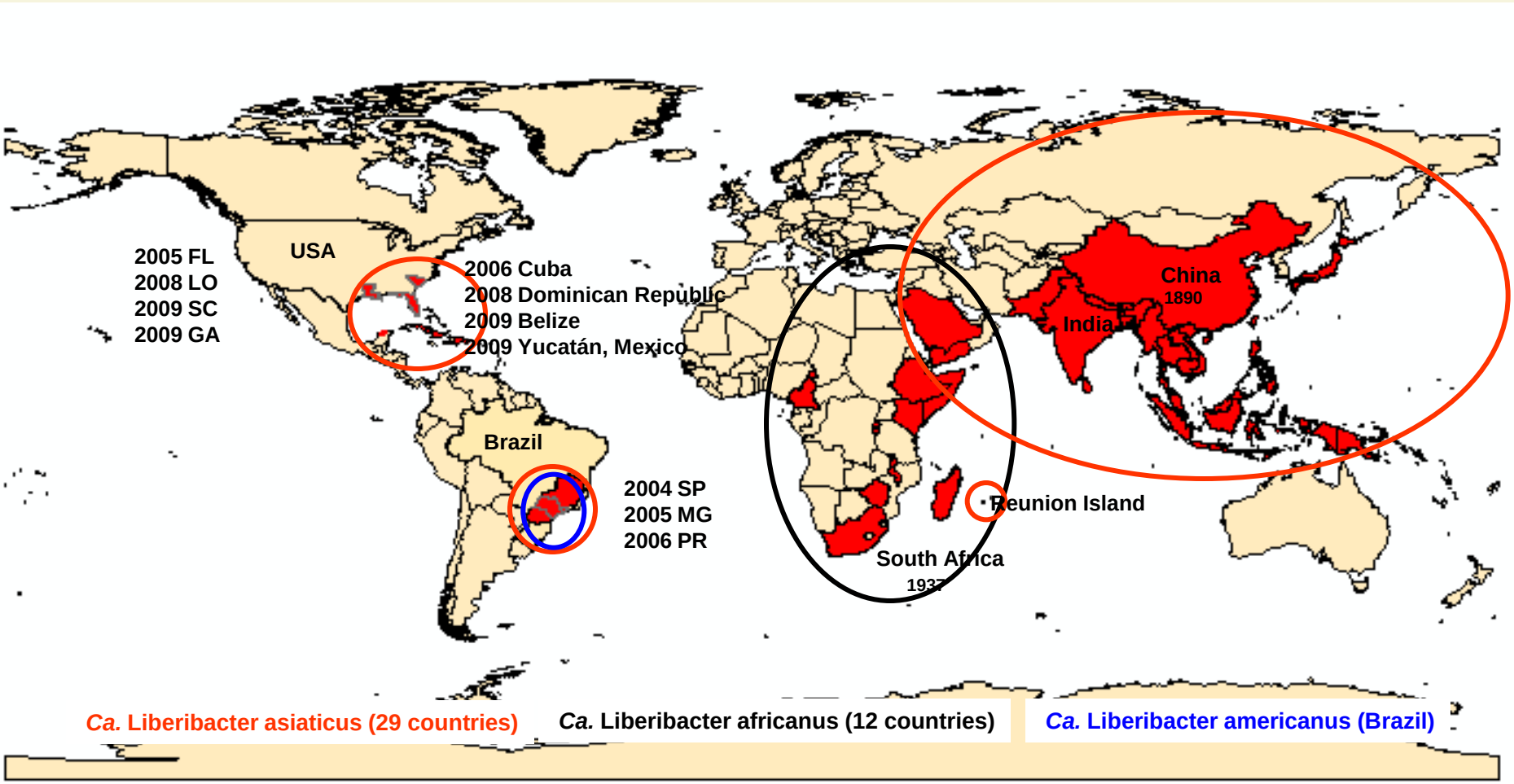
Progress on *Diaphorina citri* research

- **Feeding behavior**
- **Liberibacter transmission**
- **Temperature influence on life cycle**
- **Natural enemies**
- **Attractants and repellents**

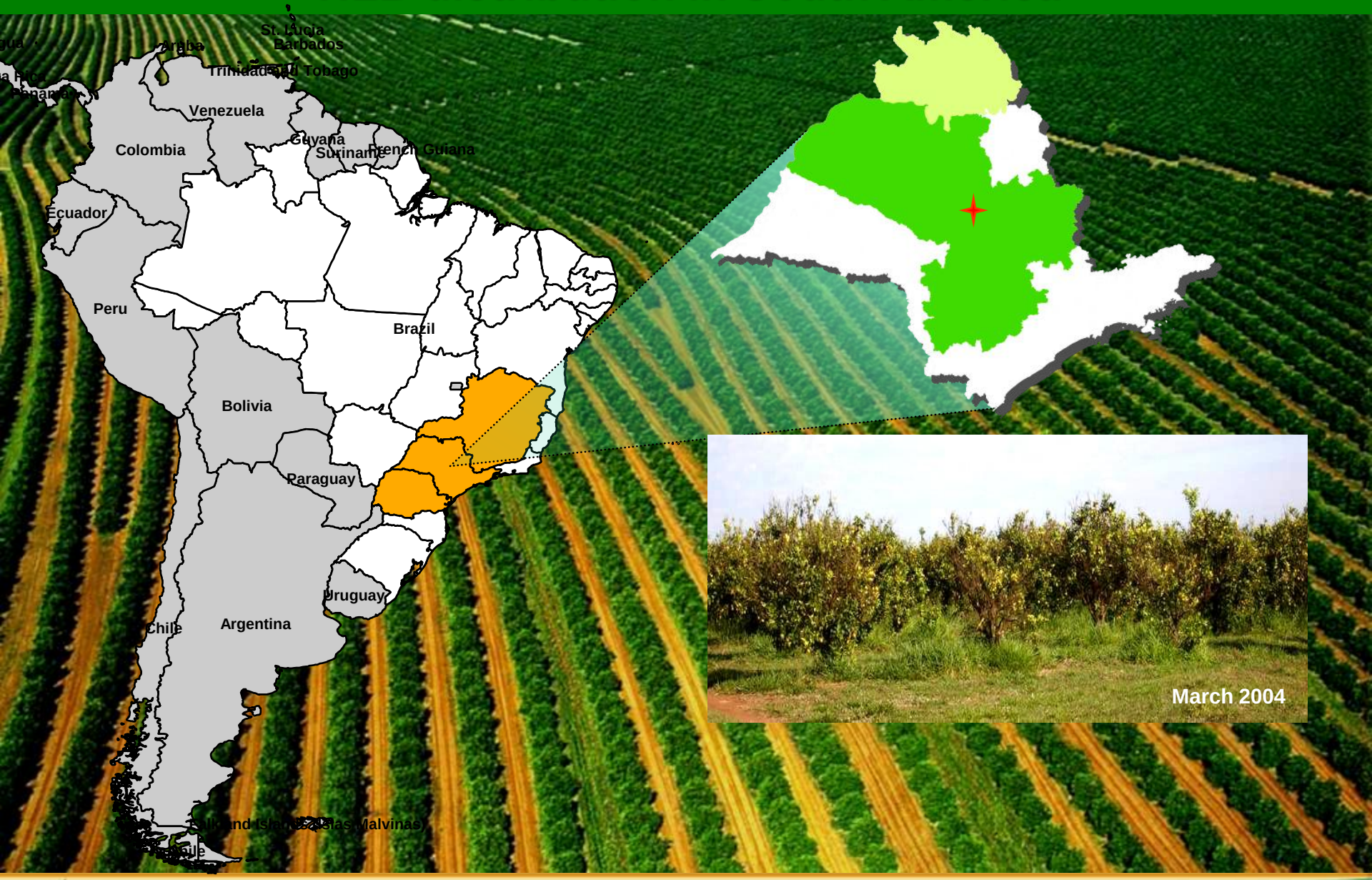
Benefits and perspectives of the research findings on HLB management

Summary and conclusion

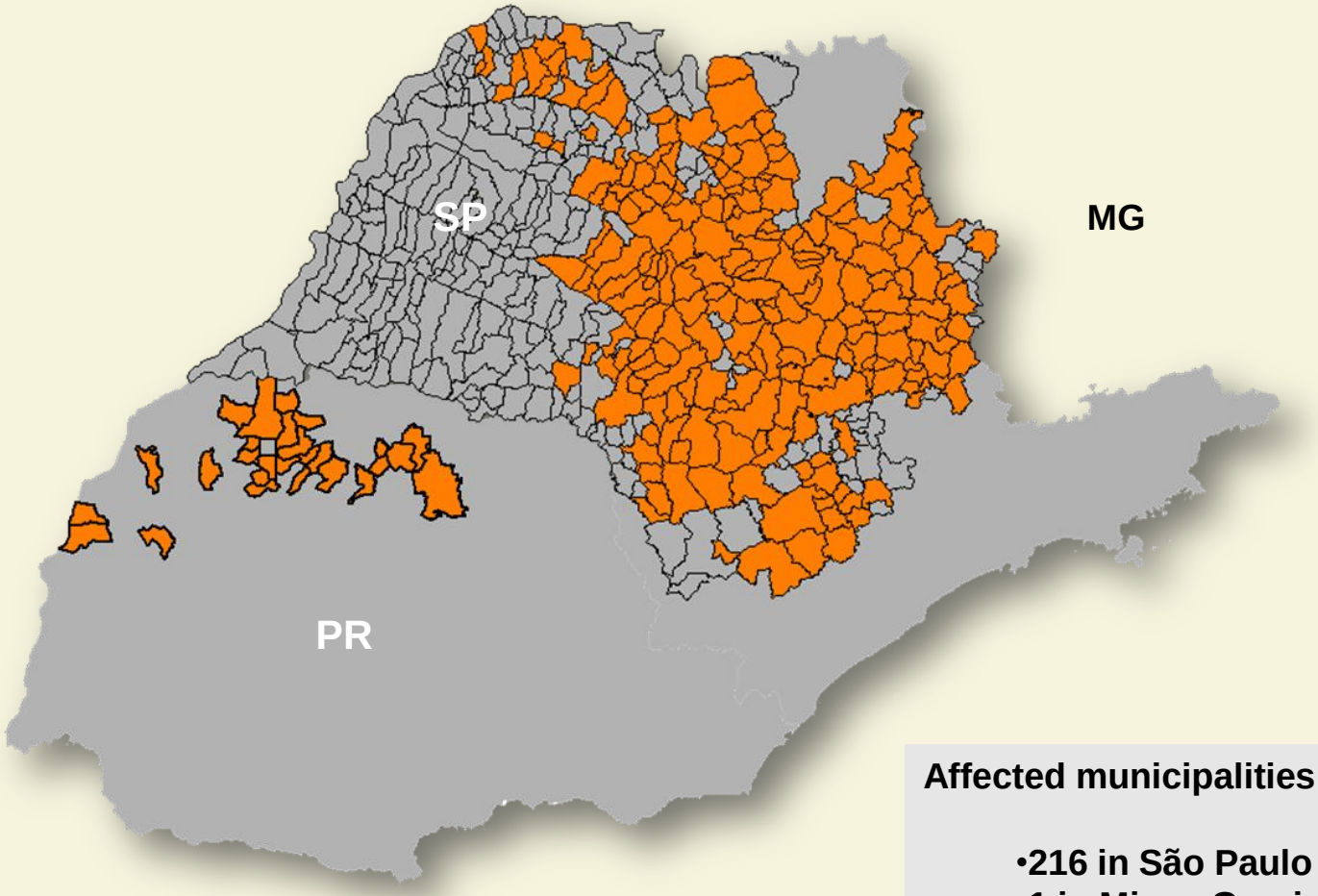
HLB distribution in the world



HLB distribution in South America



HLB distribution in Brazil



Affected municipalities (May 2009)

- **216 in São Paulo (SP)**
- **1 in Minas Gerais (MG)**
- **34 in Paraná (PR)**

Disease management practices in Brazil

Elimination of symptomatic trees

Insecticide applications

Planting healthy young trees

Presentation topics

Introduction

Progress on HLB research

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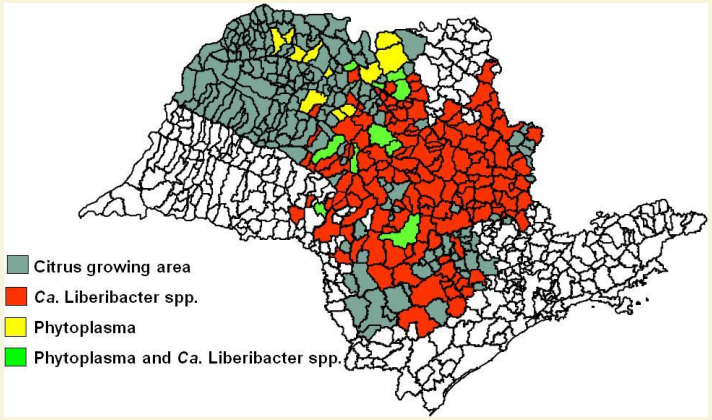
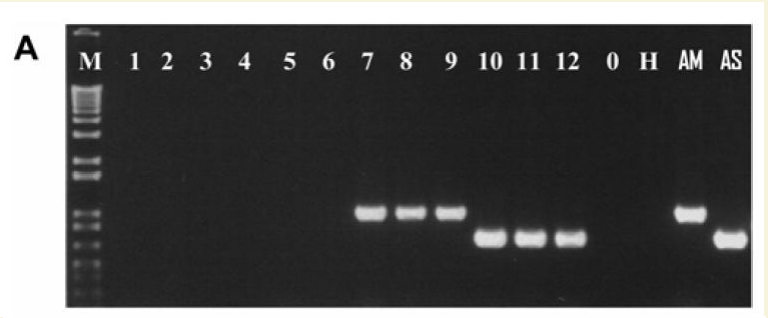
Description of a new Liberibacter species in Brazil

Teixeira et al. 2005

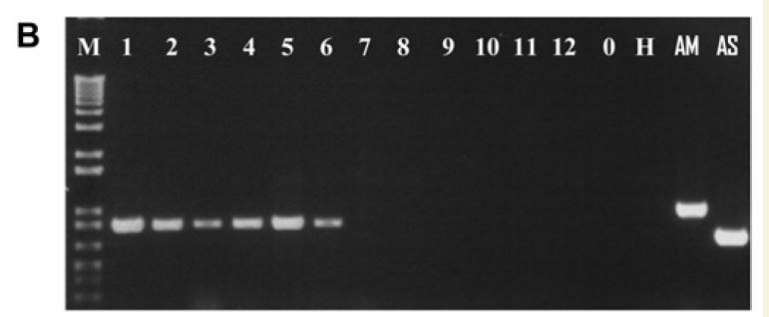
Description of two phytoplasmas associated with HLB-like symptoms



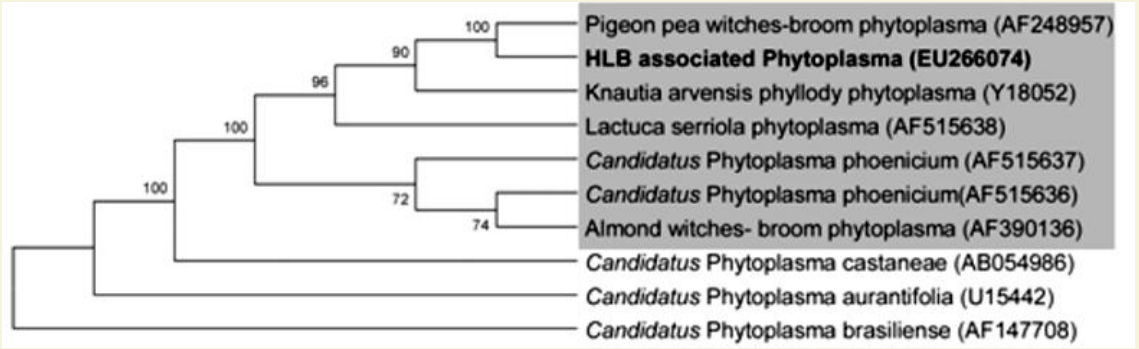
PCR specific for *Ca.* L. asiaticus or *Ca.* L. americanus



PCR specific for Phytoplasma

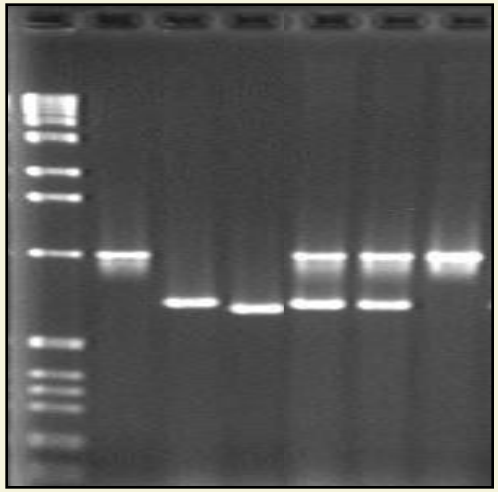


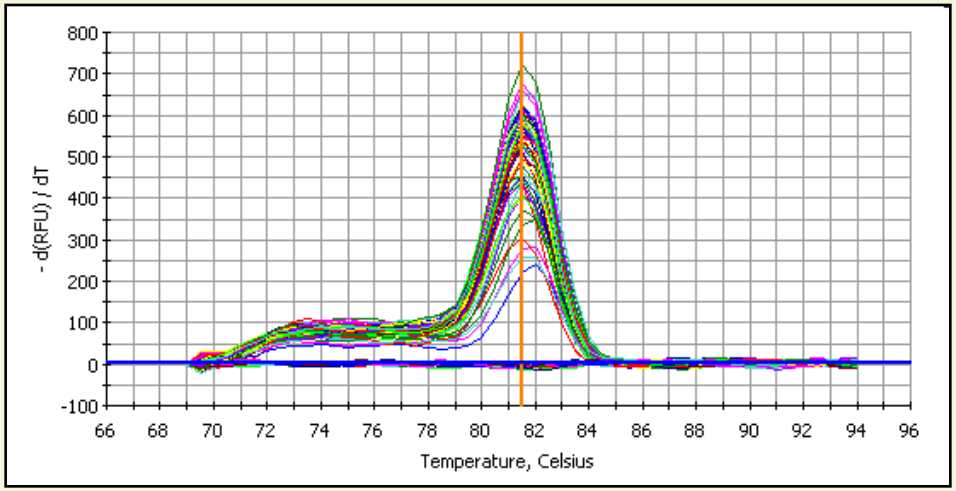
Teixeira et al. 2005



A phytoplasma related to '*Candidatus* Phytoplasma asteri' detected in citrus showing huanglongbing (yellow shoot disease) symptoms in Guangdong. P. R. China. Chen et al. 2009.

Liberibacter detection and quantification



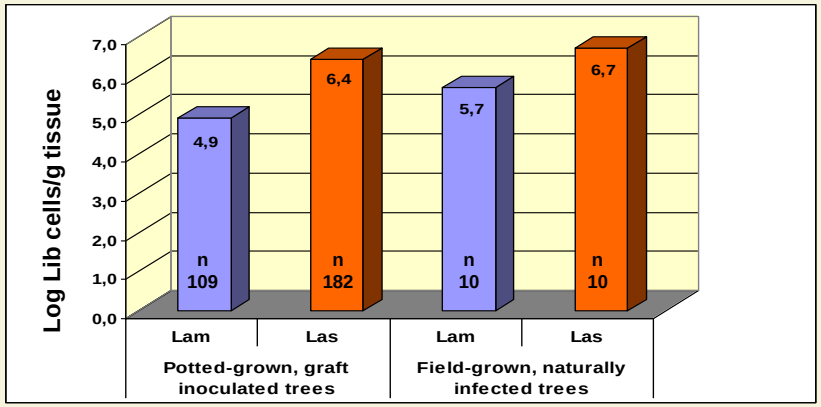


Li et al. 2006
Teixeira et al. 2008

Contrasting behavior of *Ca.* L. americanus and *Ca.* L. asiaticus in Brazil

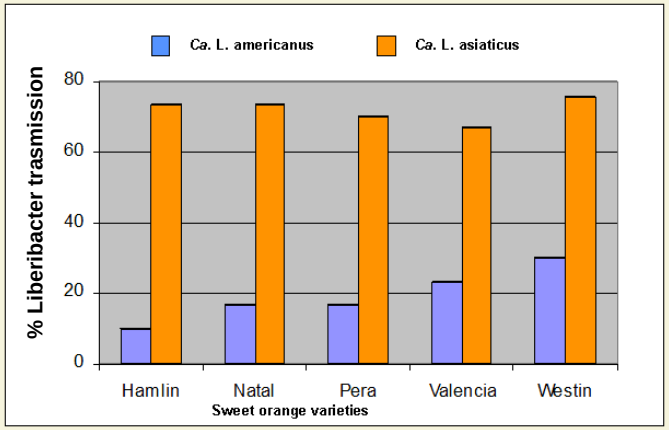
Multiplication efficiencies in citrus trees

Liberibacter populations in potted and field grown sweet oranges

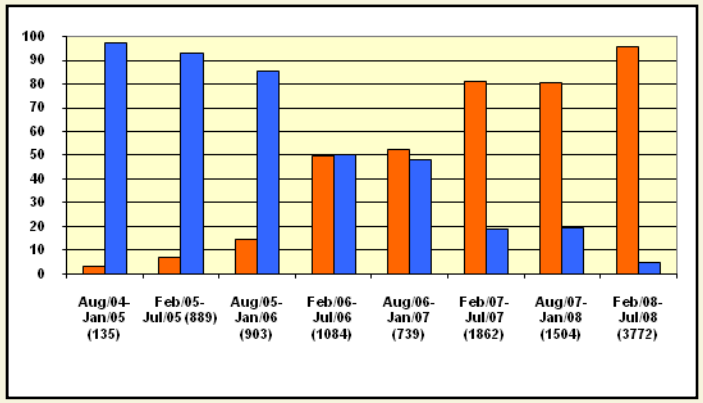


Lopes et al. 2009

Graft transmission effcienies of Liberibacter to sweet oranges

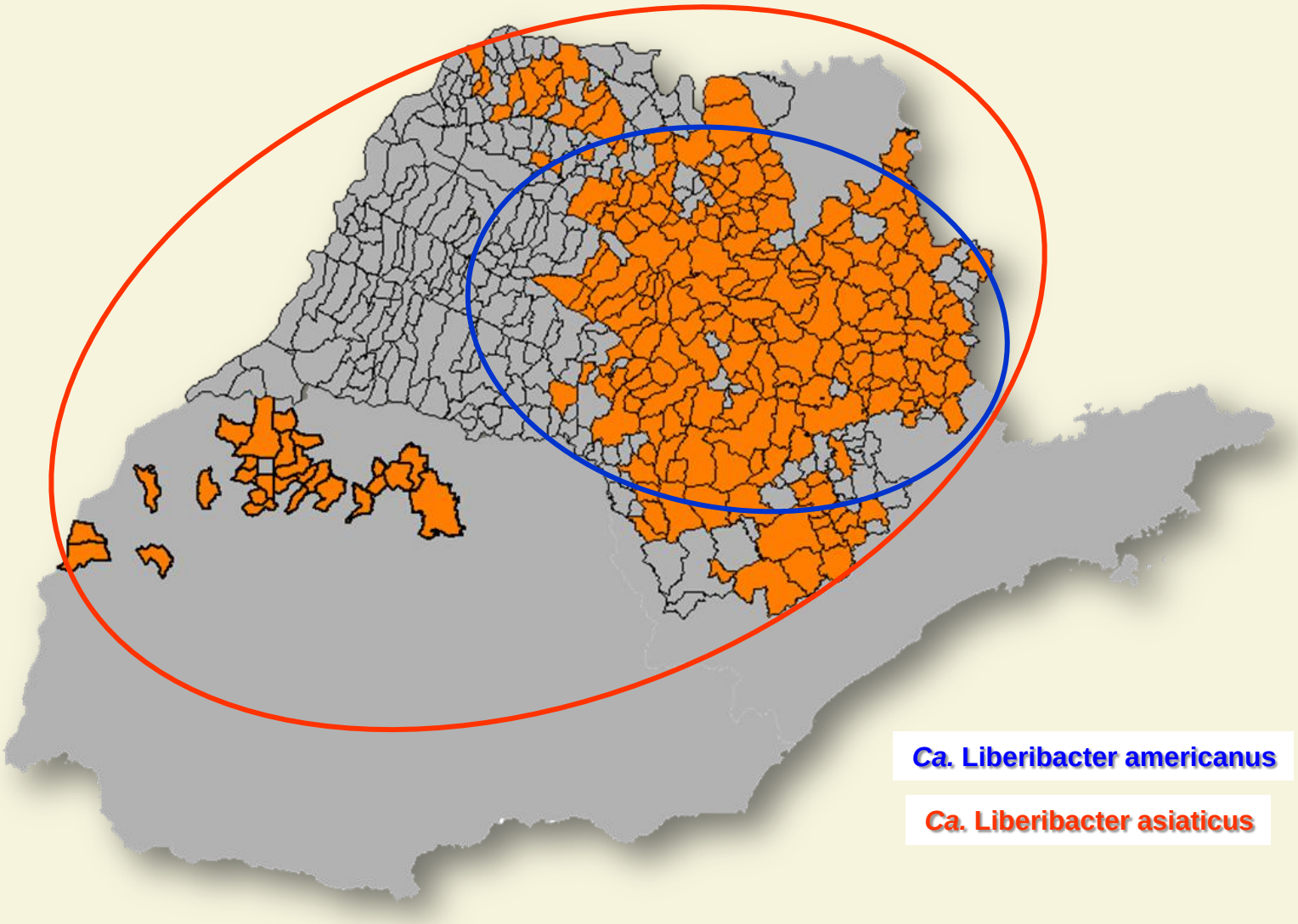


Liberibacter occurrence in São Paulo State overtime



Contrasting behavior of *Ca.* L. americanus and *Ca.* L. asiaticus in Brazil

Multiplication efficiencies in citrus trees



Contrasting behavior of *Ca.* L. americanus and *Ca.* L. asiaticus in Brazil
Sensitivity to high temperatures

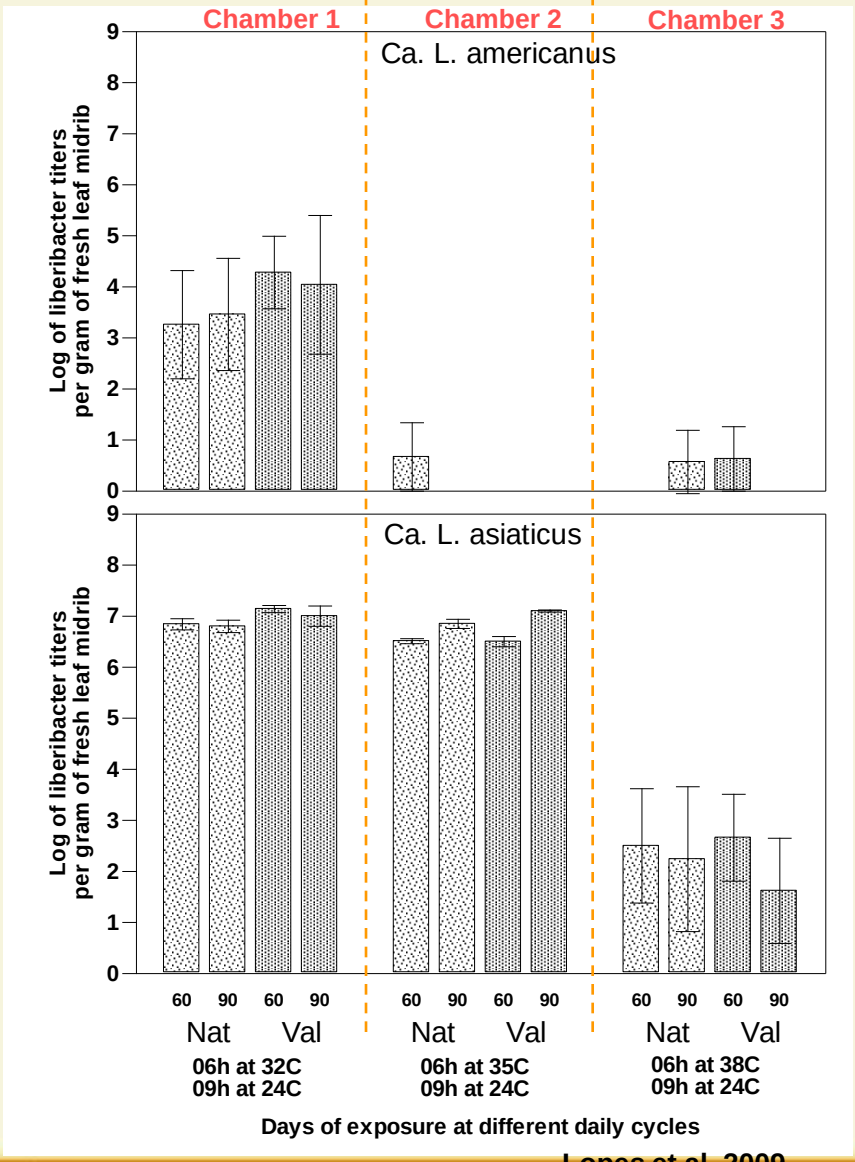
Pruned

Growth chambers

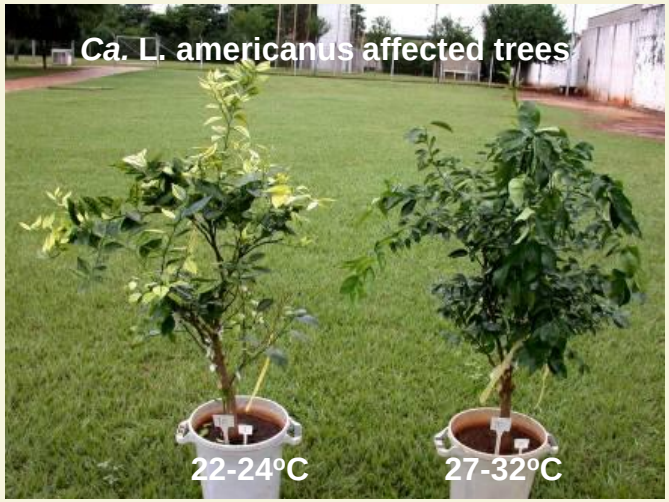
- . 22ºC - 24ºC
- . 27ºC - 32ºC
- . 24°C - 32°C (1. 9 or 12h)
- . 24°C - 32°C (6h)
- . 24°C - 35°C (6h)
- . 24°C - 38°C (6h)

Contrasting behavior of *Ca.* L. americanus and *Ca.* L. asiaticus in Brazil

Sensitivity to high temperatures



Lopes et al. 2009



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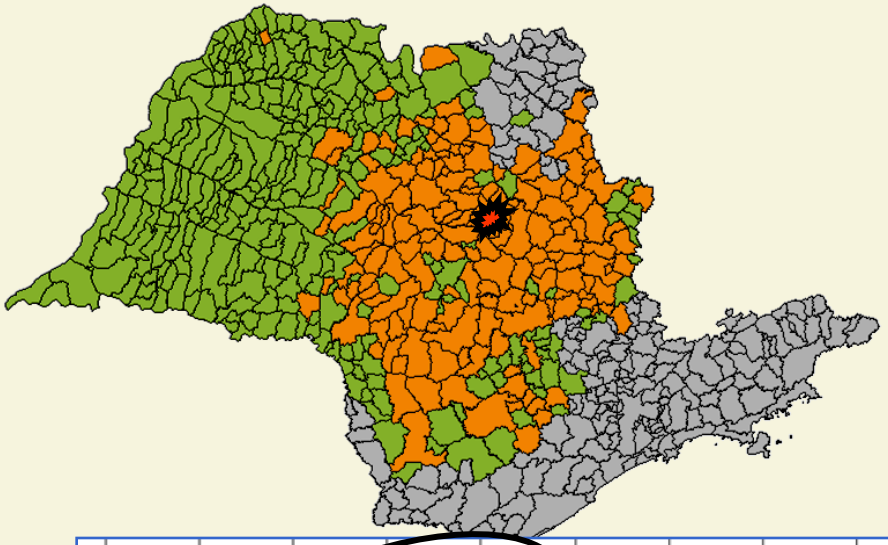
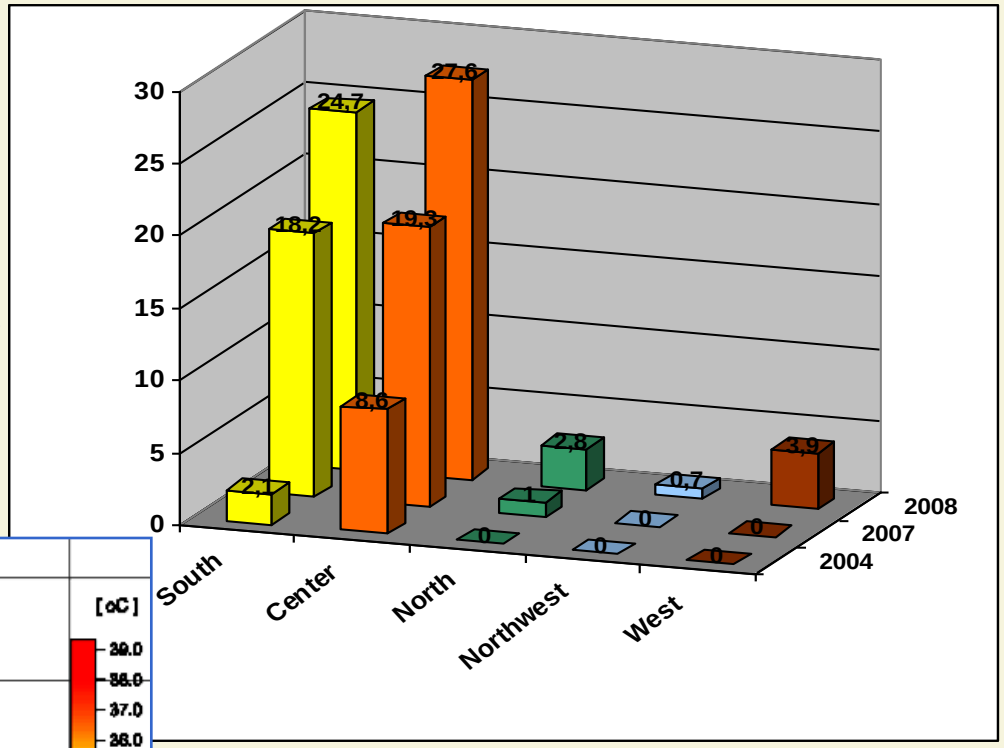
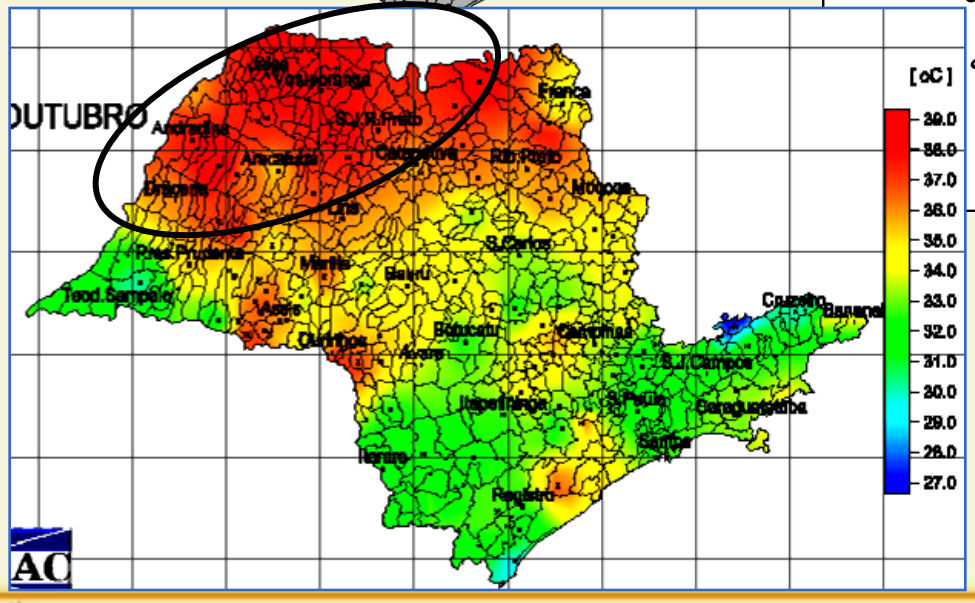
Contrasting behavior of *Ca.* L. americanus and *Ca.* L. asiaticus in Brazil
Sensitivity to high temperatures







Ca. L. asiaticus distribution in citrus trees

_Ca._ L. asiaticus

Gottwald et al. 2008

_Ca._ L. americanus

Total 216 pruned trees
Disease reappeared in 58.3%

Total 376 pruned trees
Disease reappeared in 62.5%

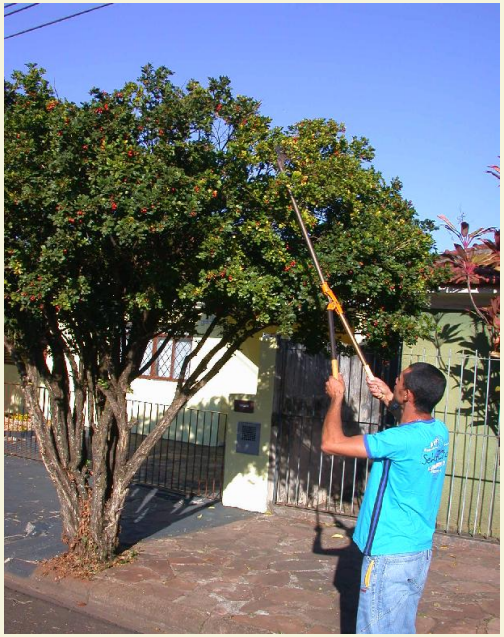
Lopes et al. 2007

Murraya paniculata as an alternative host of Liberibacter

Murraya distribution in the Araraquara region

Murraya paniculata as an alternative host of Liberibacter

Murraya survey in urban areas



Sampled 550 symptomatic trees in 17 municipalities

- **11.4% positive for *Ca.* L. americanus**
- **0.5% positive for *Ca.* L asiaticus**
- **88.1% negative**

Presentation topics

Introduction

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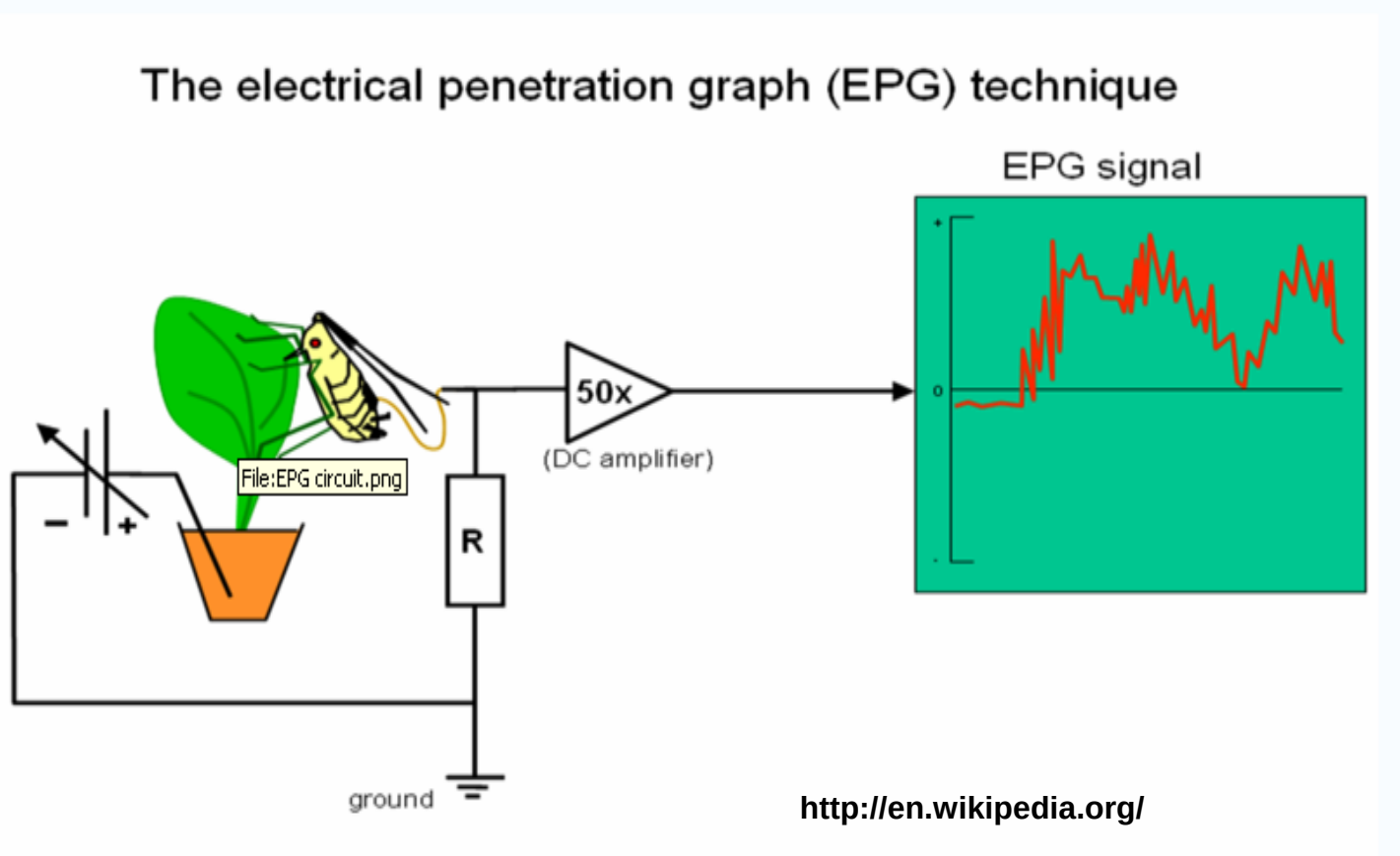
Diaphorina citri feeding behavior

Electrical penetration graphing
Tjalingii.1988



Diaphorina citri feeding behavior

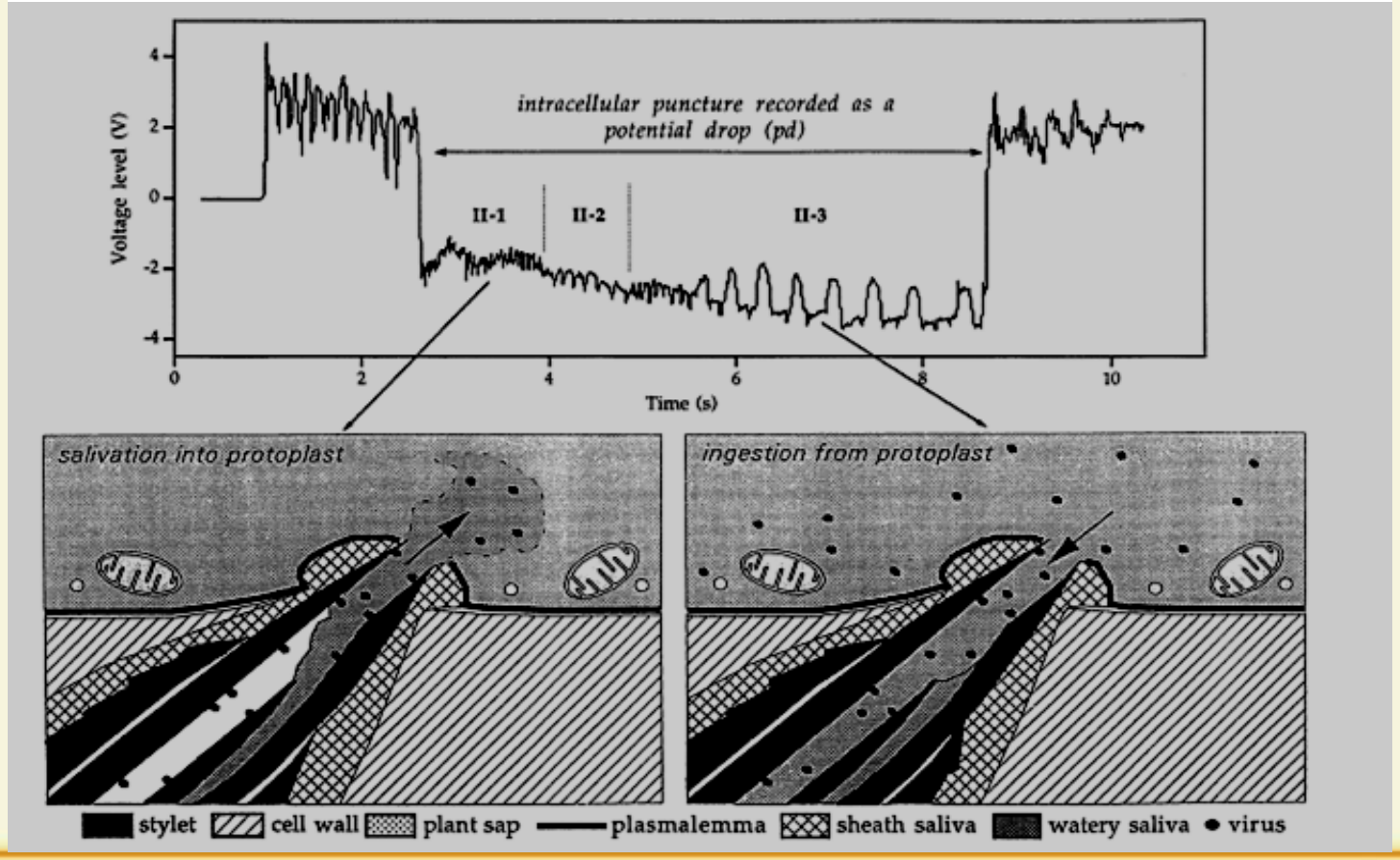
Journal of General Virology (1997), **78**, 2701–2705. Printed in Great Britain

SHORT COMMUNICATION

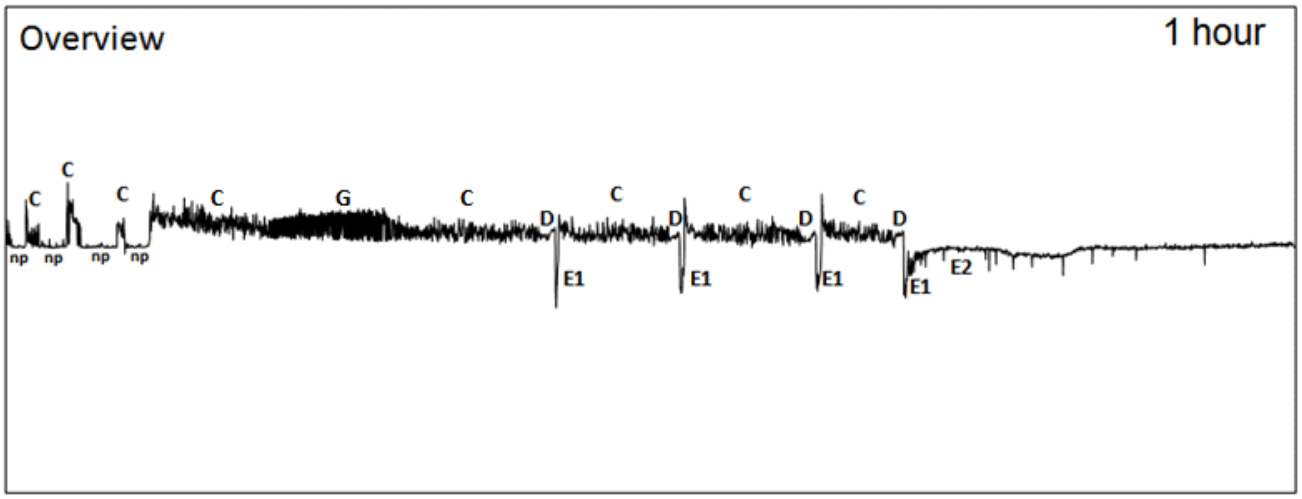
Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses

B. Martín,[1] J. L. Collar,[1] W. F. Tjallingii[2] and A. Fereres[1]

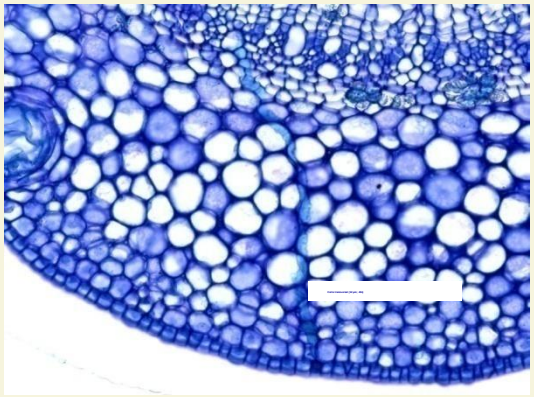
[1] Centro de Ciencias Medioambientales, CSIC, Serrano 115 dpdo., 28006 Madrid, Spain
[2] Department of Entomology, Agricultural University, POB 8031, 6700 EH Wageningen, The Netherlands



Diaphorina citri feeding behavior



Cross section of a leaf petiole showing the *D. citri* stylet pathway



Stylet activities

(i) penetration of the stylet into the intercellular parenchyma
(ii) contact of the stylet with the phloem sieve tube
(iii) salivation
(iv) phloem sap ingestion

- Average time for the stylet to reach the phloem -154 min
- Phloem sap ingestion continued for average 206.1 min over an 8 hour (240 min) recording period

Bonani et al. 2008

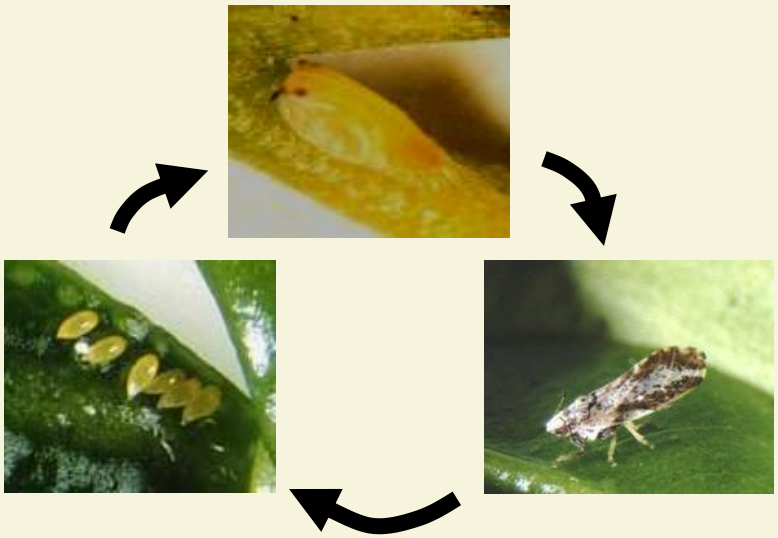
Liberibacter transmission by *D. citri*

Citrus leaf developmental stage	Young leaves	Old leaves
		
Reached the phloem (after 96 hour)	80%	20%
Ca. L. asiaticus acquisition efficiency	54%	10%

Bonani et al. 2008

D. citri developmental stage	Nymphs	Adults
		

Temperature influences on *D. citri* life cycle



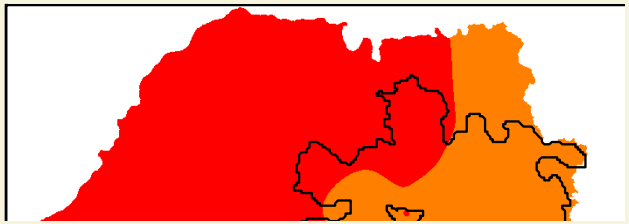
Temperature (ºC)	Pera sweet orange	
	Egg	Nimph
18	8.2 ±0.08 a	37.6±1.54 a
20	6.9±0.11 b	29.6±1.46 b
22	5.7±0.07 c	23.4±0.25 c
25	4.6±0.18 d	13.2±0.47 d
28	3.6±0.14 e	12.4±0.26 d
30	2.9±0.06 f	11.8±0.47 d
32	3.0±0.09 f	.....

Parra et al. 2007

Temperature of -1.9°C for up to 10 hours killed a relatively low percentage of adults. while -5.0 to -5.5 °C for 4 hours or longer killed 95 to 100% adults

Hall. 2008

Temperature favorability for *D. citri* in São Paulo State



Unfavorable (below 15ºC)

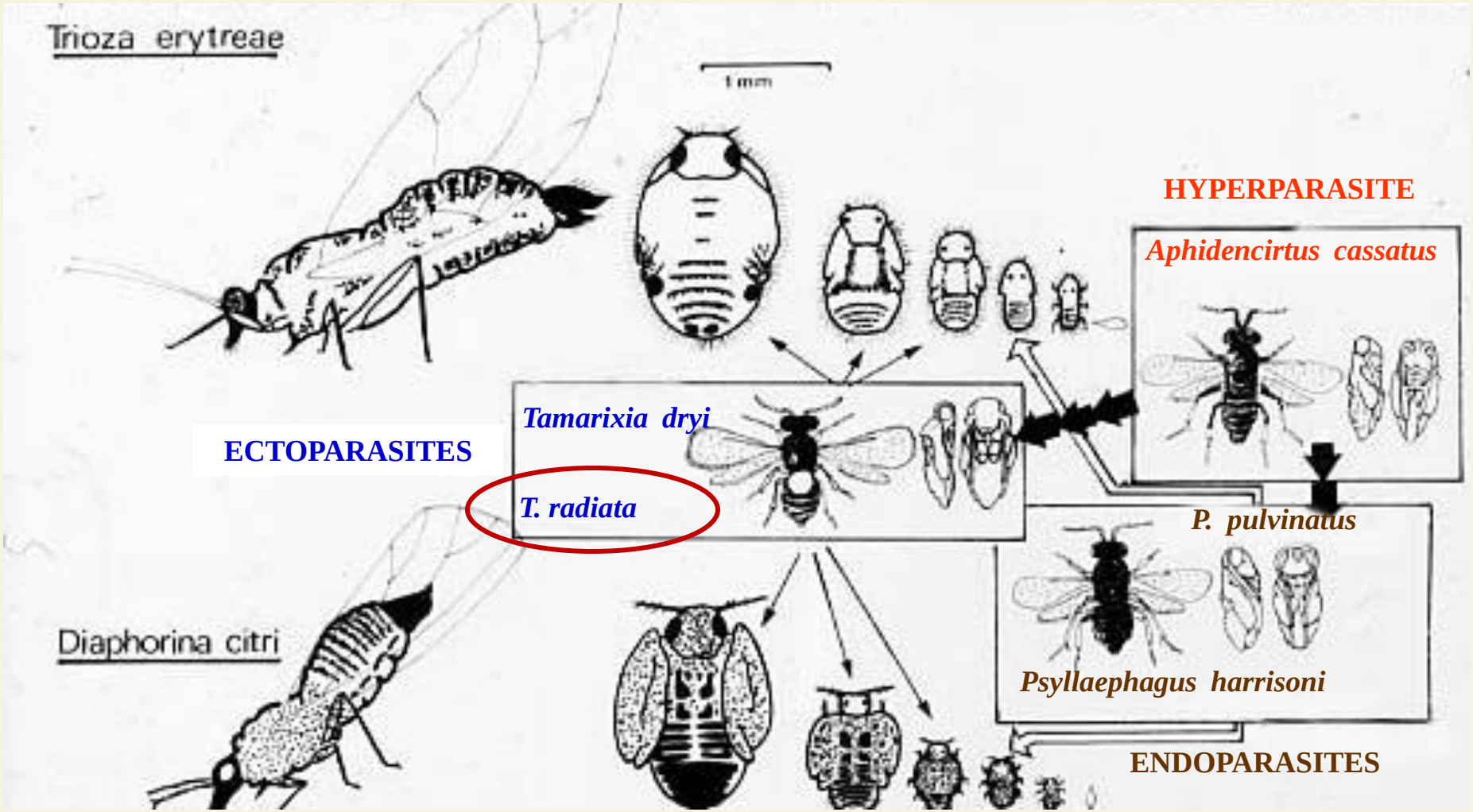
Little favorable (15ºC to 20ºC)

Favorable (20ºC to 25ºC)

Highly favorable (20ºC to 25ºC)

(Morandini et al. 2005)

D. citri natural enemies



***D. citri* parasitism rates of 27.5 to 80.0% have been observed in SP and of 56% in Florida !**

Parra et al. 2006; Qureshi et al. 2009

D. citri natural enemies

Tamarixia

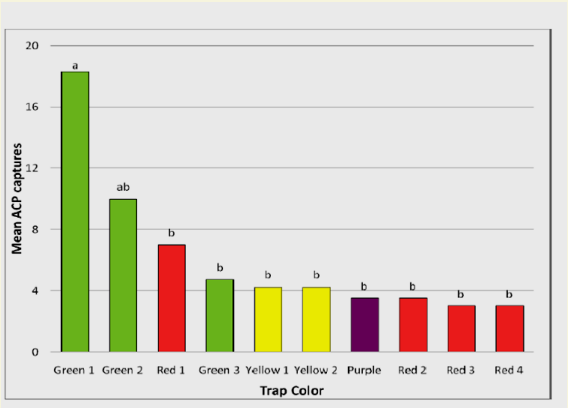
***D. citri* parasitized nymphs**

Non-parasitized nymphs

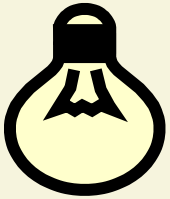
D. citri attractants and repellents

Attractants

Wenninger et al. 2009



Sétamou et al. 2008



Repellent

Zaka and Zeng. 2008

Ishinose et al. 2008

wounded guava leaves

dimethyl disulfide

Rouseff et al. 2008

Presentation topics

Introduction

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Summary and conclusion

Benefits and perspectives - PCR in the HLB diagnosis

PCR advantages

- High sensitivity
- High specificity
- Estimation of bacterial titers

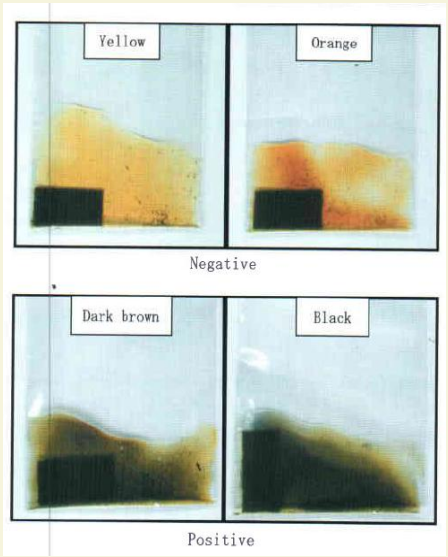
PCR disadvantages

- Costly reagents
- Costly thermo-cyclers and centrifuges
- Time consuming
- Specialized personnel
- Detect live and dead cells

Use limited to

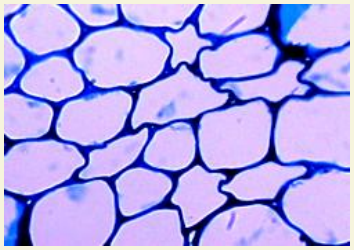
- Research
- Train inspectors for symptom recognition
- Confirmation of HLB in leaf samples with questionable symptoms

Benefits and perspectives - Iodine test in field diagnosis

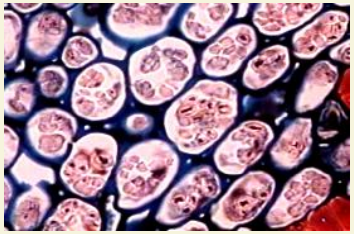


Takushi et al. 2007

Parenchyma cells of healthy leaf



Parenchyma cells of mottled leaf



Heredia et al. 2006

In Florida, the iodine and qPCR tests agreed for 76% of the samples

The test is useful for field diagnosis but unsuitable as a substitute for PCR confirmation

Chamberlain and Irey 2008

Benefits and perspectives - Field identification of symptomatic trees

48% efficiency

60% efficiency

Belasque 2006

Benefits and perspectives
The role of asymptomatic citrus as source of inoculum

Plant number	5 Months			6 Months			7 Months		
	Symptom[w]	Ct[x]	Titer[y]	Symptom	Ct	Titer	Symptom	Ct	Titer
'Ca. L. americanus'									
1	mt, –	22.09	5.79	mt, –	24.12	6.24	–, df	23.88	6.24
2	mt, df	27.70	4.34	mt, df	24.43	5.92	mt, df	25.04	5.63
3	mt, –	27.56	3.95	mt, –	23.06	6.28	mt, df	26.68	5.17
4	mt, –	21.75	6.16	mt, –	26.98	5.13	mt, df	27.32	5.12
5	–, df	37.06	1.49	–, df	23.98	6.16	mt, df	25.47	5.51
6	–, –	–	–	–, –	–	–	–, –	32.49	3.51
7	–, –	–	–	–, –	–	–	–, –	34.17	2.91
8-10	–, –	–	–	–, –	–	–	–, –	–	–
Average	…	27.23	4.35	…	24.51	5.95	…	27.86	4.87
'Ca. L. asiaticus'									
1	mt, –	17.77	7.47	mt, –	18.75	7.31	mt, df	20.51	7.20
2	mt, –	17.61	7.42	mt, –	17.93	7.44	mt, df	20.11	7.22
3	mt, df	17.52	7.36	mt, df	17.92	7.44	mt, df	19.78	7.47
4	–, df	19.36	6.78	–, df	18.74	7.27	mt, df	19.97	7.34
5	mt, –	19.76	6.97	–, df	18.77	7.13	–, df	20.57	7.15
6	–, –	20.17	6.39	–, –	19.26	6.77	–, df	20.85	7.05
7	–, –	17.67	7.29	–, –	18.70	6.94	–, df	20.51	7.09
8	–, –	22.41	6.17	–, df	19.12	7.45	–, df	19.51	7.13
9	–, –	21.55	6.50	–, –	22.22	6.37	mt, df	21.06	6.95
10	–, –	17.66	7.42	–, –	21.62	6.64	mt, df	20.82	6.84
Average	…	19.15	6.98	…	19.30	7.08	…	20.33	7.15

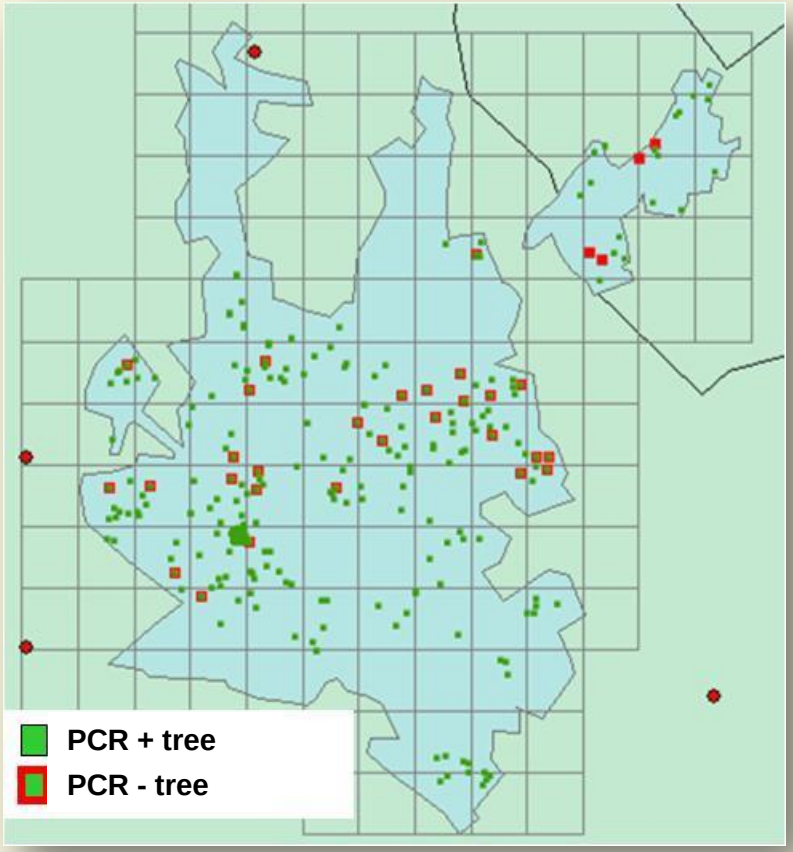
Symptomatic

Asymptomatic

mt = mottling df = mineral defficiency

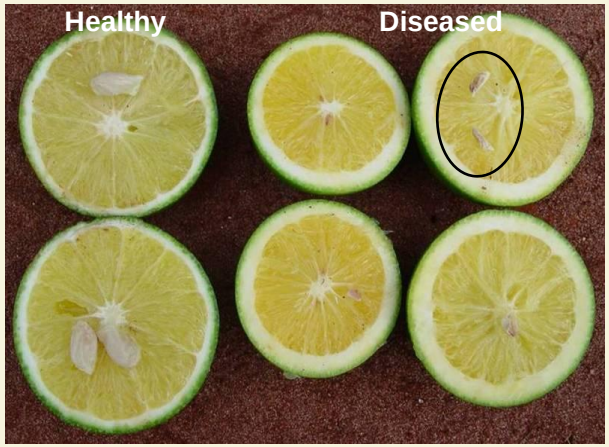
Benefits and perspectives
The role of *Murraya paniculata* as source of inoculum

Distribution of PCR + and - trees of *M. paniculata* in the city of Araraquara, SP

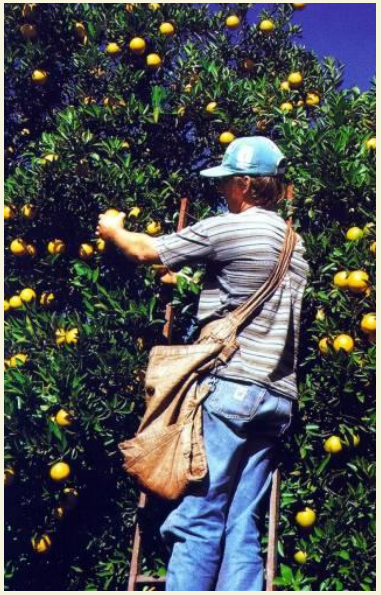


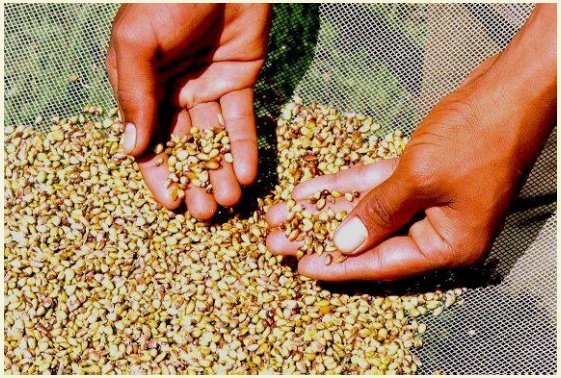
Bassanezi et al

Benefits and perspectives - The question of Liberibacter seed transmission

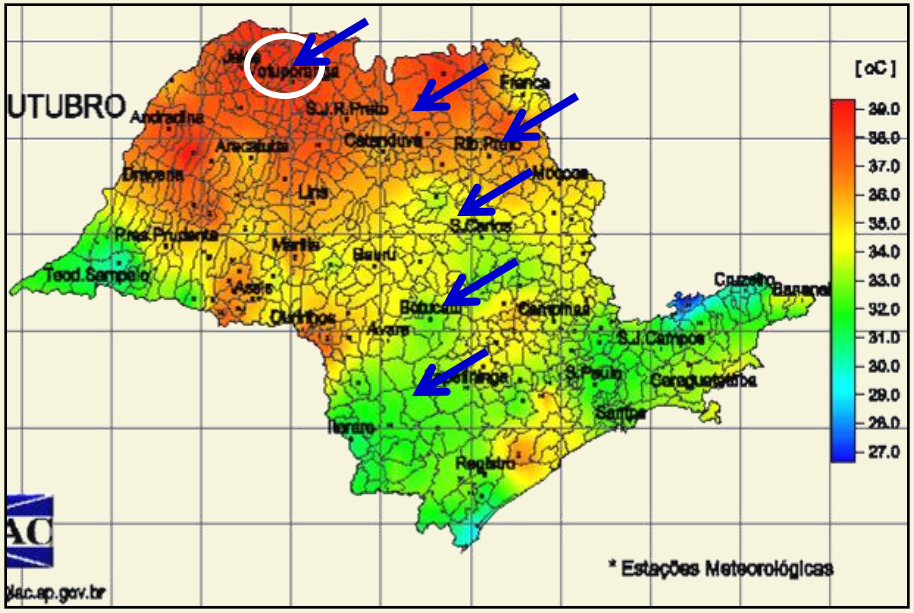


Authors	Transmission to seedlings	Detection method	Symptoms
Graham et al, 2008	2006 7/59 → 3/7 → 1/3	qPCR	No information
	2007 6/723	qPCR	No information
	2008 5/290	qPCR	No information
Hartung et al, 2008	1/89	qPCR	Stunting, defoliation, chlorosis
Zhou et al, 2008	14%	qPCR	Atypical (vein yellowing, leaf curling) only when stressed by nutrient deficiency

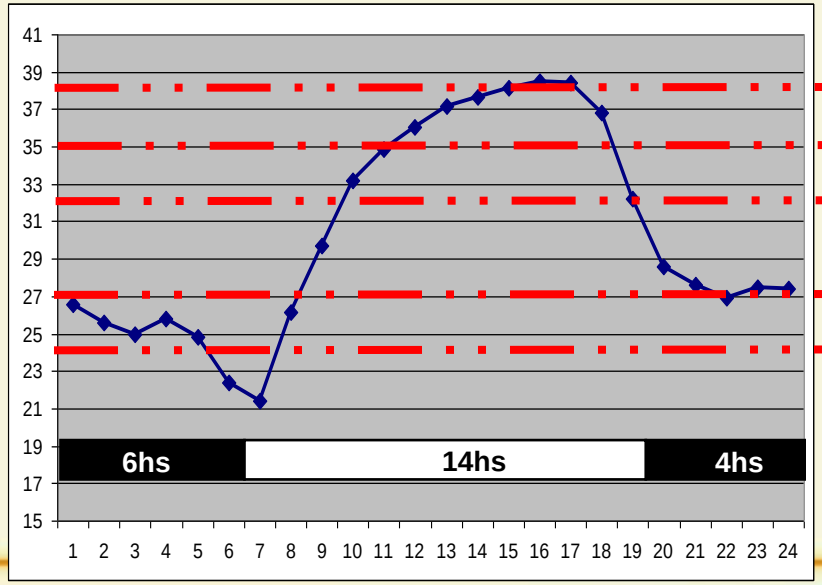




Benefits and perspectives
The question of Liberibacter sensitivity to high temperatures



A typical hot summer day in Votuporanga, NW of SP



Liberibacter multiplication in citrus

	Am	As
→	-	- (+)
→	-	++
→	+/-	++
→	++	++
→	++	++

Growth chamber experiments

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Benefits and perspectives
D. citri attractants and repellents

Guava/citrus inter-planting
Not practical in Brazilian commercial groves

Synthesized repellant compounds to be released slowly in the field ?

A transgenic citrus tree repellant to *D. citri* ?

Another repellant plant species not competing with citrus to cover citrus fields ?

Presentation topics

Introduction

Progress on HLB research

Progress on *Diaphorina citri* research

Benefits and perspectives of the research findings on HLB management

Summary and conclusion

Summary and Conclusion

Main research progress in the last 5 years

- ✓Three additional bacteria have been found associated with the HLB-like symptoms
- ✓More sensitive detection methods have been developed
- ✓The ornamental *M. paniculata* was found infected with both liberibacters in Brazil
- ✓The competitive relationship of *Ca.* L. americanus and *Ca.* L. asiaticus was defined
- ✓Multiplication and distribution of Liberibacters in citrus trees were demonstrated
- ✓Feeding behavior, biology and population dynamics of the insect vector, as well as it attractiveness or repellency to visual cues, and to volatiles released from host and non host plants, were determined

Greater understanding of how the host-pathogen-vector relationship works

- ✓Improvements of management practices
- ✓Expectation for the development of new practices
- ✓Most promising areas are
  - synthesis of volatiles that repel *D. citri*
  - development of transgenic citrus trees that resist pathogen attack or prevent vector transmission

Summary and Conclusion

Muchas Gracias