



HLB- Regional Program of Control and Prevention

COSAVE REGION (ARGENTINA-BRASIL- CHILE
PARAGUAY-URUGUAY-BOLIVIA)

Ing. Agr. Ms.Sc. Pablo Cortese

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What is Huanglongbing (HLB)?

Ethyologic agent: *Candidatus Liberibacter* spp.

Phloematic Bacteria Gram (-),

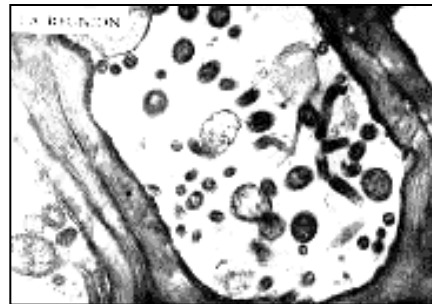
Phylum: Proteobacteria

Class: Alphaproteobacteria

Order: Rhizobiales

Family: Phyllobacteriaceae

Gender: *Candidatus Liberibacter* spp



From Silvio Lopes Fundecitrus Brazil

Species:

Candidatus Liberibacter asiaticus

Candidatus Liberibacter americanus

Candidatus Liberibacter africanus

Host:

Citrics (Citrus spp.); ornamentals (Ex. *Murraya* sp.)



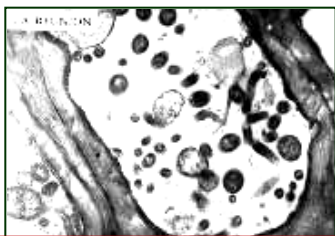
The HLB epidemics is a interaction among the plant, the bacteria and the vector insects



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All citrics



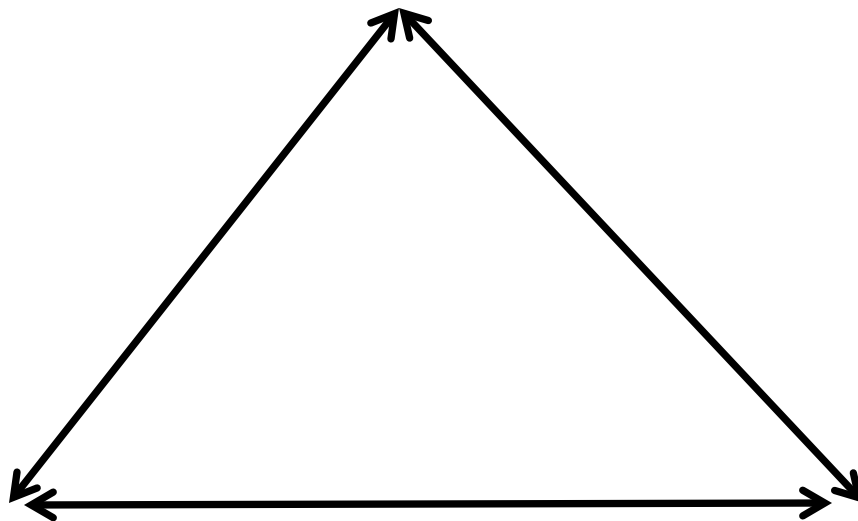
- Candidatus Liberibacter americanus*
- Candidatus Liberibacter asiaticus*
- Candidatus Liberibacter africanus*



Diaphorina citri



Trioza erytreae





SAVE

DL

does not
the de-
of HLB

The most destructive disease of citrus in the world

A Case Study of Huanglongbing (Greening) Control in Reunion

B. Aubert, M. Grisoni, M. Villemin, and G. Rossolin

ABSTRACT In an effort to reduce the serious effects of citrus huanglongbing (HLB) (greening) over the past 25 years in Reunion, citrus growers were supplied with disease-free plants and biological control of the psyllid vectors was implemented. A survey was conducted in 1995 in the area of Petite Ile which represents all the features of citrus cultivation in Reunion. A mere 0.5% of the over 6,000 orchard trees surveyed, ranging in age from 3 to 25 years, were found to have HLB symptoms, clearly illustrating the success of the program.

Citrus huanglongbing (HLB) (greening) and its two psyllid vectors, *Trioza erytreae* (Del Guercio) and *Dia-phorina citri* Kuwayama, are known to threaten seriously citrus crops of Reunion and the neighbouring islands of Mauritius and Madagascar (3, 4, 7, 8). The devastating effect of the disease was demonstrated by an extensive epidemiological study conducted over an 8 years period (1971-79) in Reunion, for assessing the

ing selected budwood of SRA origin under insect-free conditions while the latter was finalized by importing and acclimatizing exotic natural enemies, especially the parasitic wasps *Tamarixia radiata* Waterston and *Tamarixia dryii* Waterston (2). This dual approach combining clean policy nursery and integrated vector control has proven to be effective in re-establishing profitable domestic citrus production.



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Its the most destructive disease of citrus in the world

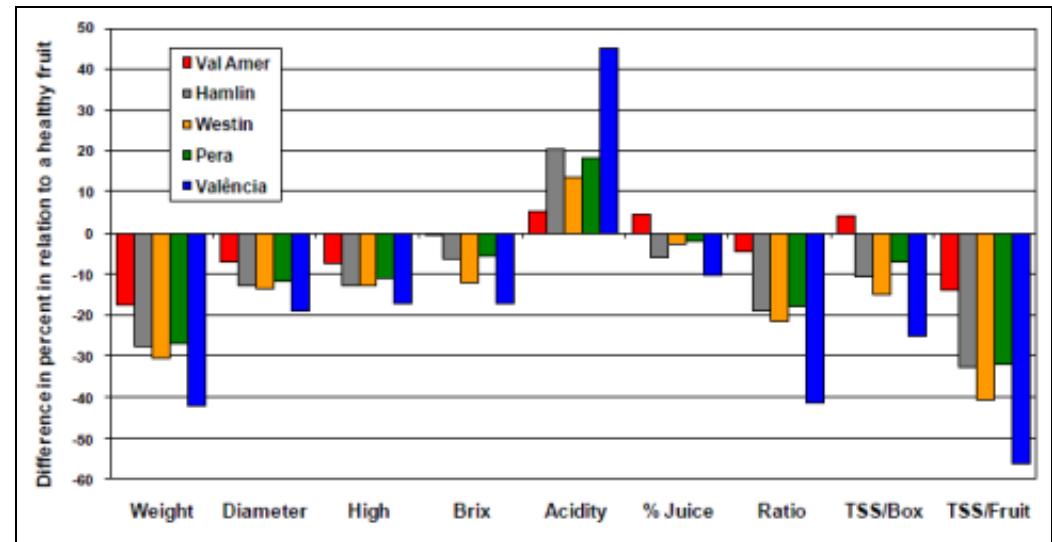
After the infection:

Young plants don't reach economic production and died in a short time (6 months to a year)

Older plants are not productive in 4-5 years (depending on productive conditions) and finally died

Main damages

- Fruit drops
- Fruit with less weight
- Less sugar level
- Increase of acid level
- Less juice percentage
- Color and shape alterations
- Decline and death of plants in 2 or 3 years



Bassanezi et al, 2009.

Over 100 million trees already eradicated in the world



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Country	Losses	Year / period
Philippines	7 million trees	1962
Indonesia	3 million trees	1960-1970
South Africa	30-100% some areas	1932-1946, 1958
Brazil (São Paulo)	22 million trees (approx.)	2004-2012

Da Graça,
1991

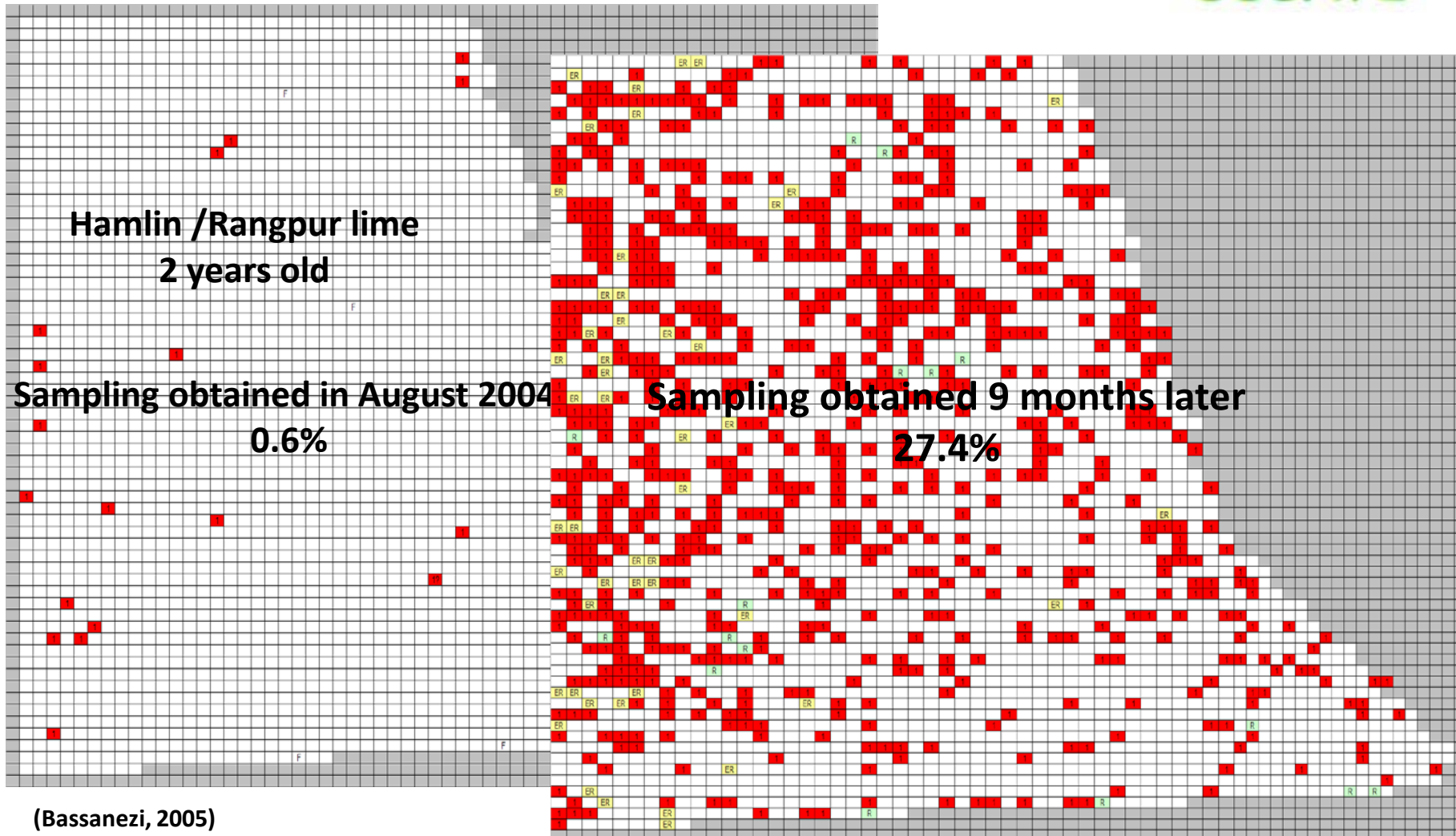


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The development of the disease can be dramatic

Very much affected surrounding plot

Very much affected surrounding plot



(Bassanezi, 2005)



Citric propagation material
Host plants

Psellids





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Liberibacters associated with HLB

Liberibacters make up a different group of Gram – pholematic bacteria

From Silvio Lopes Fundecitrus Brazil

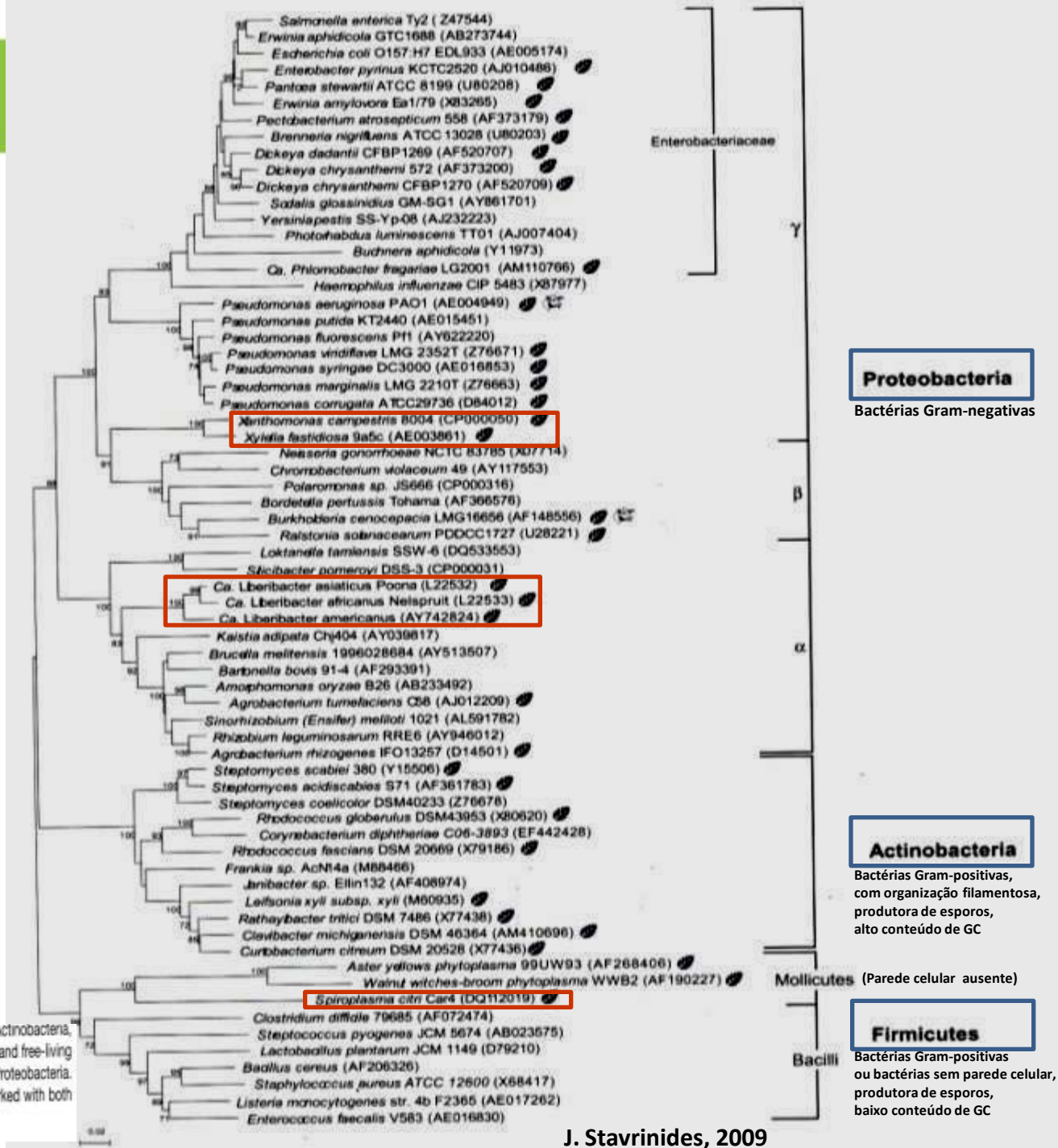


Figure 1. Phylogenetic distribution of the major plant pathogens among the Proteobacteria, Actinobacteria, and Firmicutes. Plant pathogens (denoted by a leaf) are interspersed with animal pathogens and free-living bacteria, with the vast majority of described phytopathogenic bacteria being present in the Proteobacteria. Cross-kingdom pathogens *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* are marked with both a leaf and an animal symbol.



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Liberibacters are phloematic and move throughout the plant

TABLE 1. Quantification of '*Candidatus Liberibacter asiaticus*' from different parts of huanglongbing-infected citrus trees by quantitative polymerase chain reaction assay^a

Tissue	No. of samples tested	No. tested positive	'Ca. <i>Liberibacter asiaticus</i> ' cells/ μ g of total DNA	
			Mean \pm SE	Population range
Root	8	8	10,331 \pm 5,398 B	1,837–47,414
Leaf midrib	8	8	6,693 \pm 3,528 B	159–27,666
Petal	8	6	707 \pm 446 B	44–2,889
Pistil	8	6	3,189 \pm 1,523 B	14–8,047
Stamen	6	4	1,177 \pm 699 B	337–3,253
Peduncle	7	7	67,764 \pm 16,559 A	18,364–13,7031
Columella	7	7	4,279 \pm 1,291 B	383–9,347
Seed coat	7	7	2,576 \pm 758 B	174–5,081
Endosperm	7	0	0	0
Young whole fruit	8	7	1,873 \pm 1,222 B	53–9,200
Bark	6	6	16,639 \pm 6,837 B	254–45,499

^a Bacterial populations are presented as the mean of independent assays followed by the standard error (SE) of mean. Different letters indicate statistically significant differences ($P < 0.05$). Differences between different tissues were determined by SAS (SAS, Cary, NC) using the general linear model procedure, and significantly different means ($P < 0.05$) were separated by the LS means method.

Tatineni et al, 2008

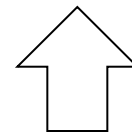
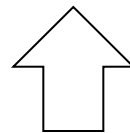
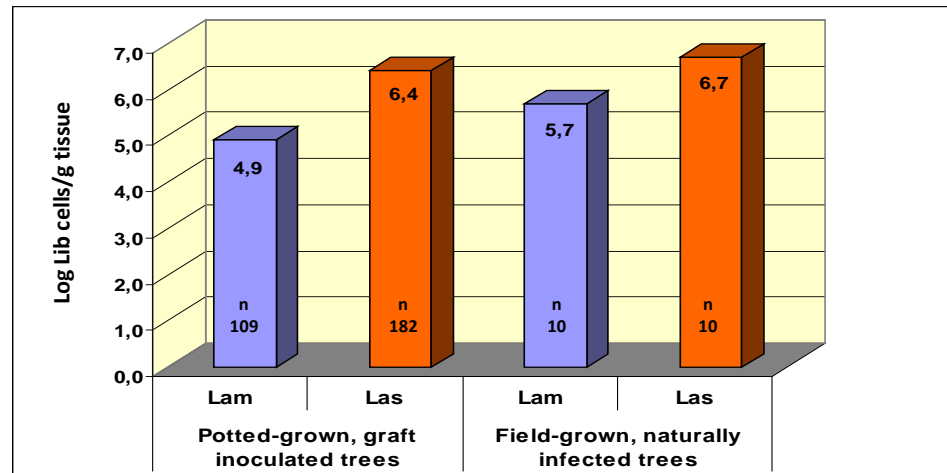


Gottwald et al., 2008



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Ca. *L. asiaticus* has higher titer than *L. americanus* in citric plants



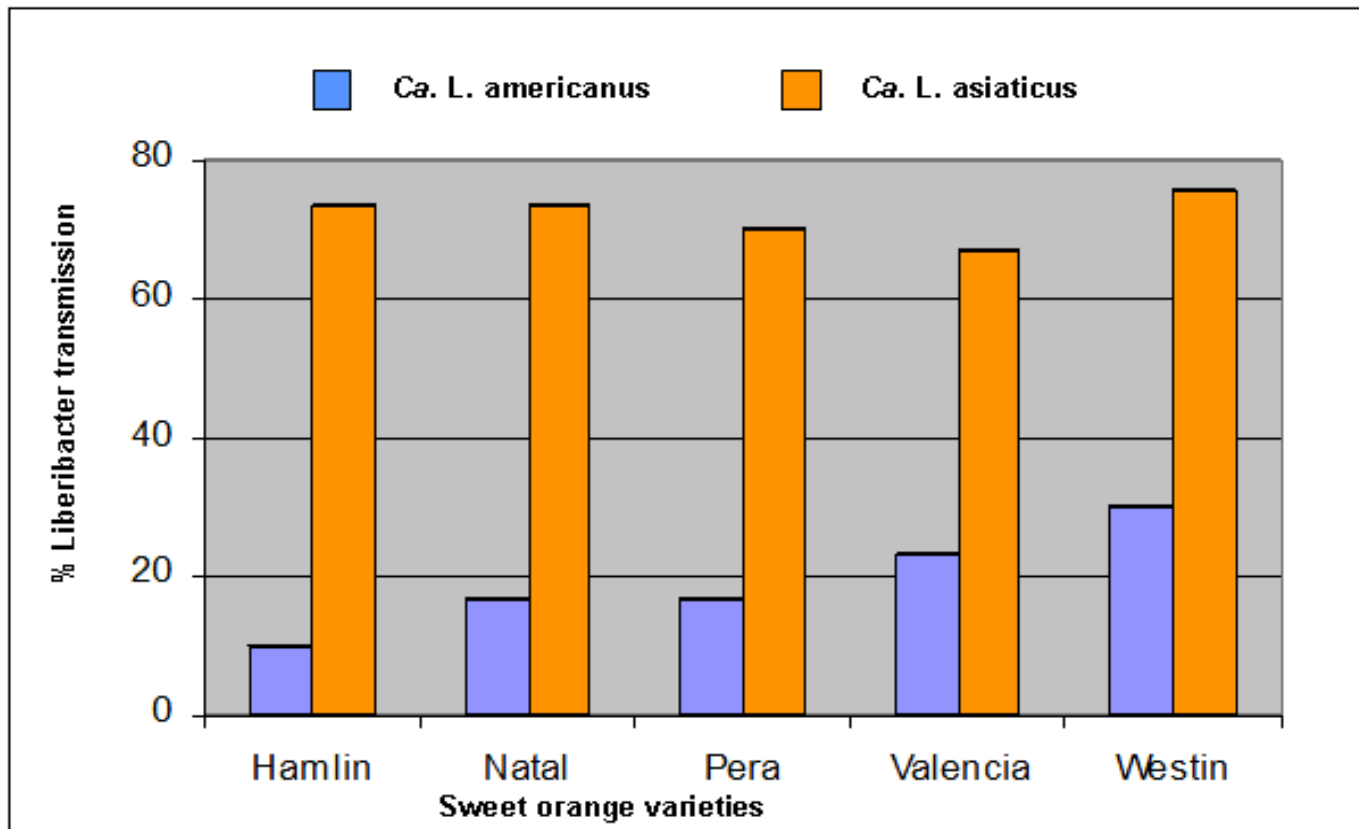
Lopes et al. 2009



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Higher titer *Ca. L. asiaticus* helps to explain:

Ca. L. asiaticus is more easily transmitted than *Ca. L. americanus*

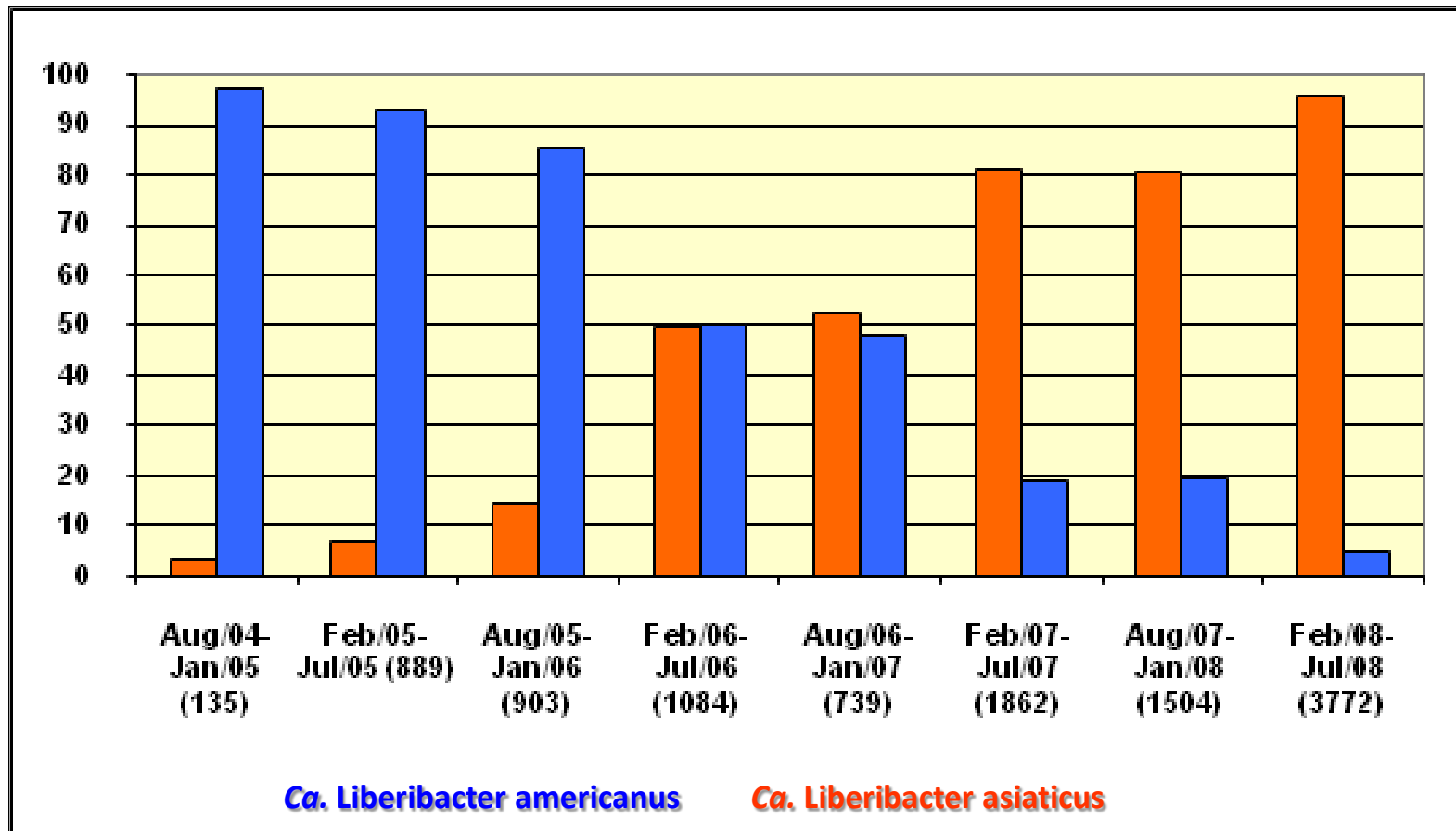




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Higher titer *Ca. L. asiaticus* (Las) helps to explain:

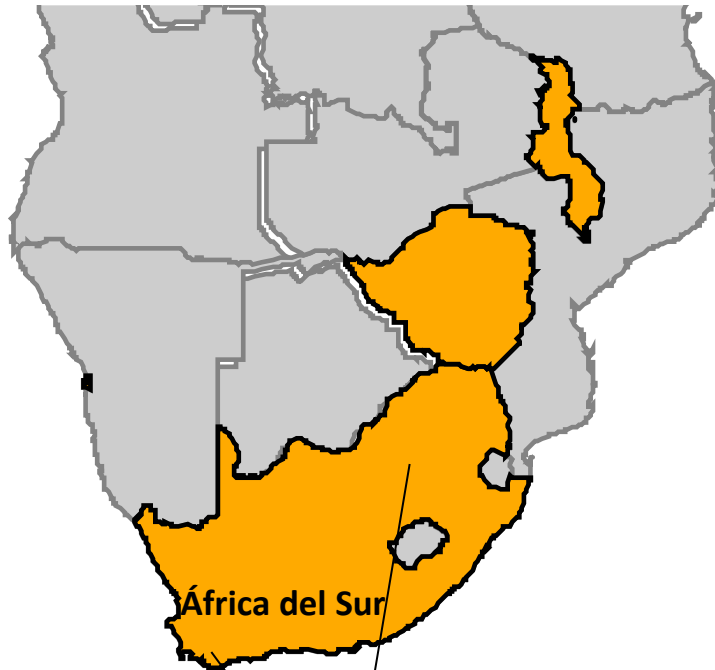
Las higher prevalence in Brazil





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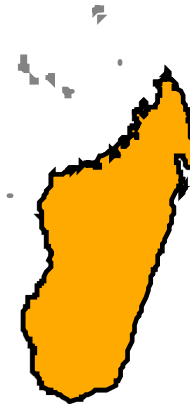
Ca. Liberibacters are sensitive to high temperatures



África del Sur

Ca. L. africanus y *T. erythrae*

The disease and his vector are more prevalent in mild temperature regions.



Islas Reunión

Ca. L. asiaticus y *D. citri*

. Present up to 500 m of altitude

Ca. L. africanus y *T. erythrae*

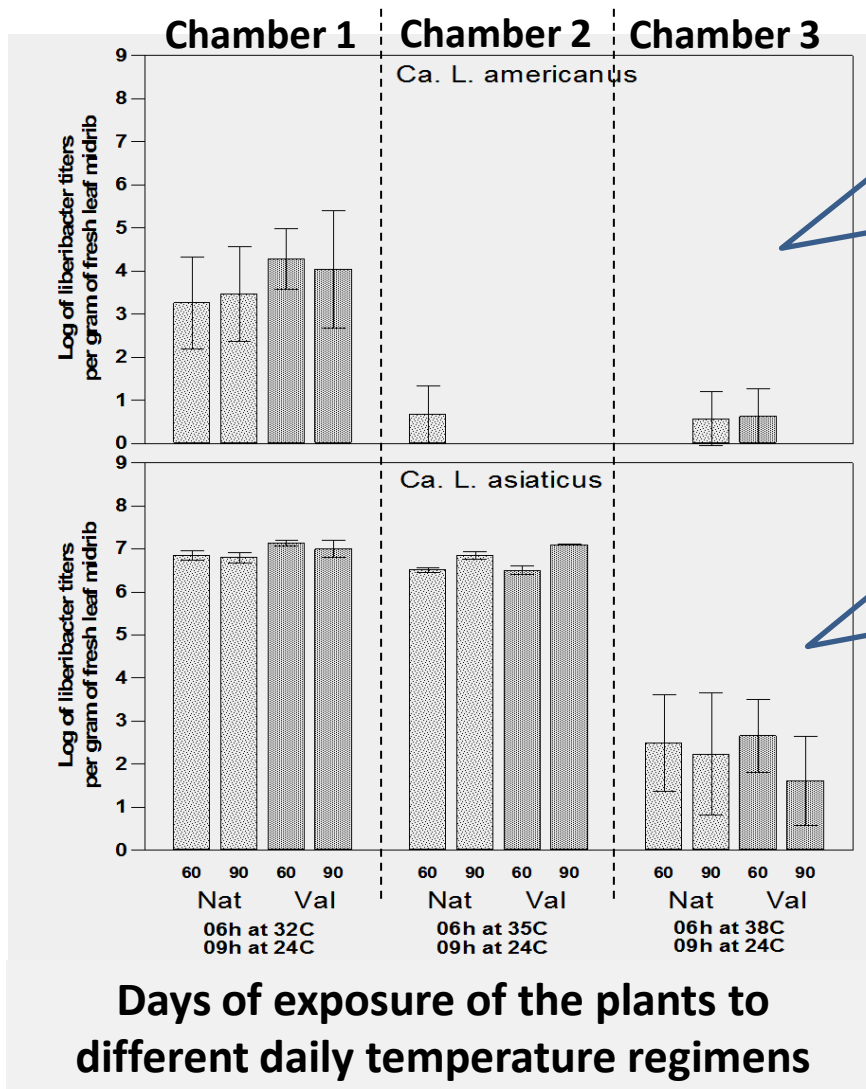
. Present in higher areas

Schwarz & Green 1972 - Bove et al, 1974



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High temperature effects



Lam multiplication and symptoms expression are negatively affected at 32°C, 35°C y 38°C.

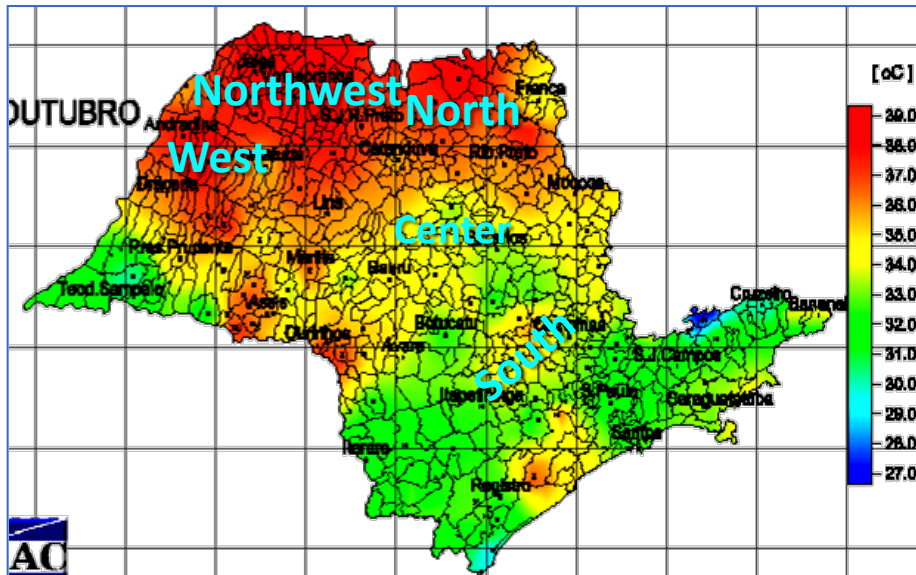
Las multiplication and symptoms expression are negatively affected only at 38°C.



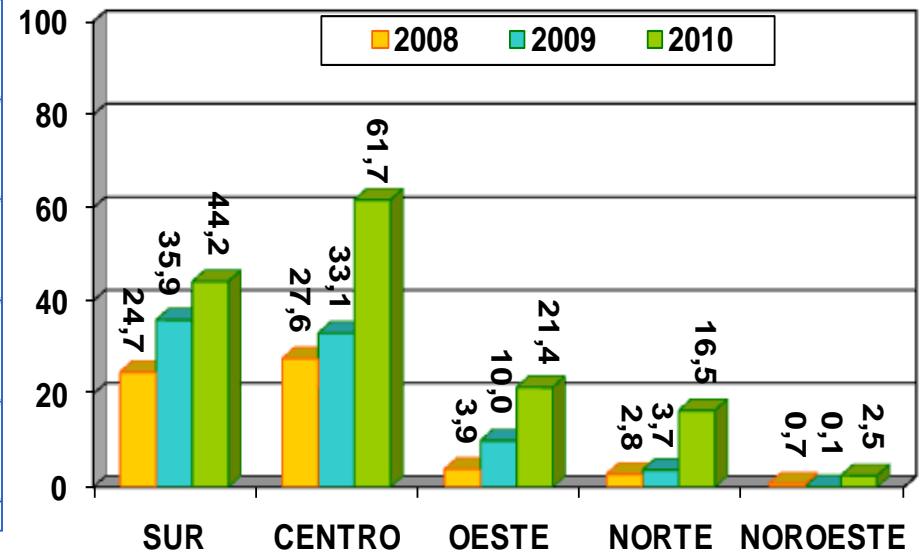
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High temperatures *Liberobacter* spp. sensitivity helps to explain: The lower dispersion of HLB in warmer summer regions in Brazil

Maximum absolute temperatures registered in São Paulo State in October 2004



Percentage of affected blocks per region





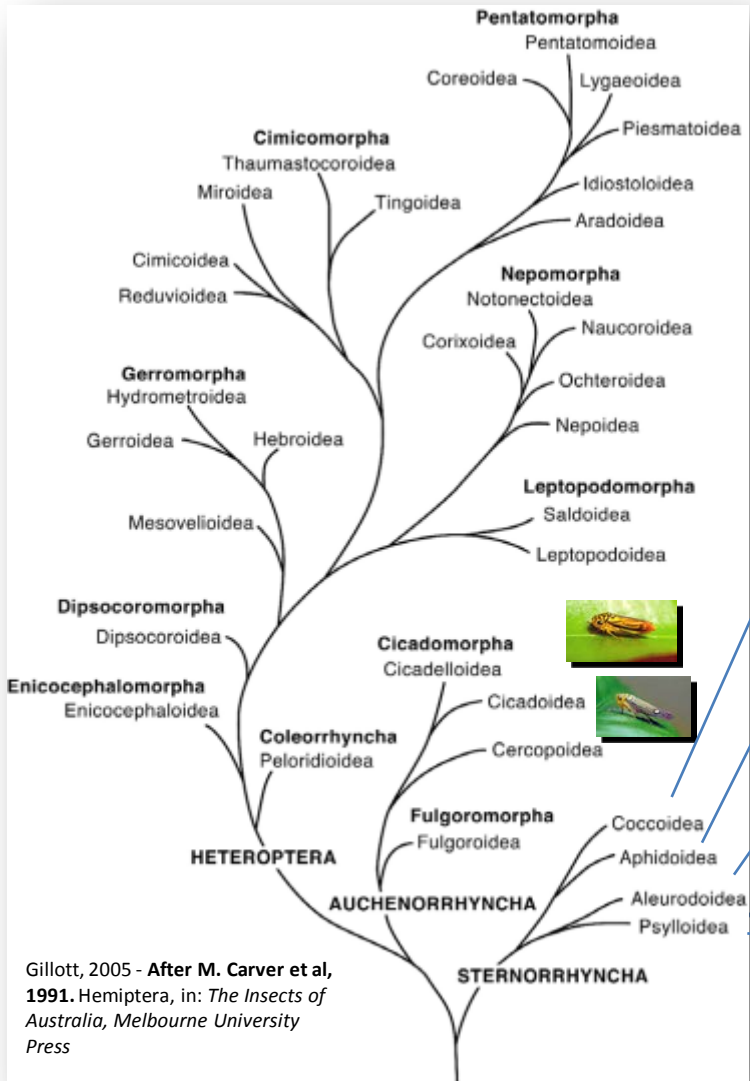
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Vector insects of Liberibacter



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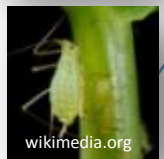
Psyllids feed on the phloem



Gillott, 2005 - After M. Carver et al, 1991. Hemiptera, in: *The Insects of Australia*, Melbourne University Press



Diaphorina citri



Trioza erytreae

From Silvio Lopes Fundecitrus Brazil

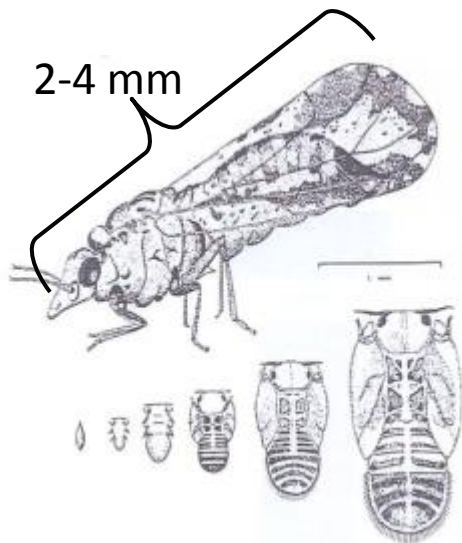
Psyllids



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- *Trioza erytreae* (present in Africa; related to *C. L. africanus*)
- *Diaphorina citri* (present in Asia and America; related to *C. L. asiaticus* y *C. L. americanus*)

Candidatus Liberibacter spp. Persistent and non propagative



Cycle of life (egg-nymph-adult): 13 - 47 days (28 a 15 °C)

Egg: 2,6 to 8,2 days

Nymph 1-5: 10,7 to 39,3 days

Adults: 3 to 4 month longevity

Source: M. Miranda - FUNDECITRUS

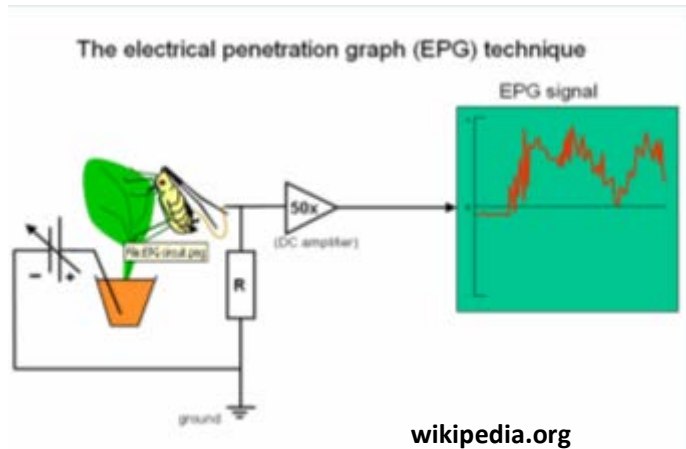




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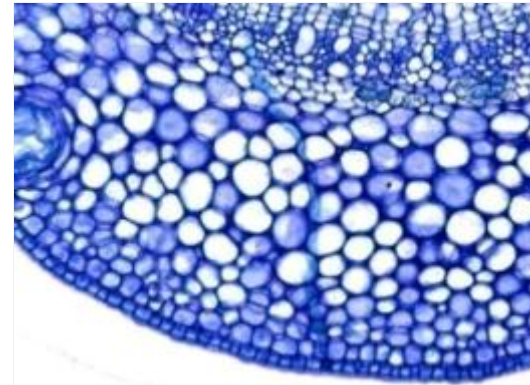
D. citri transmits Liberibacter during phloem feeding process

From Silvio Lopes Fundecitrus Brazil



Tjalingii.1988

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



Bonani et al. 2008

- Average time to reach the phloem : 154 min
- Average feeding time: 3 to 4 hours

Las can be acquired by second, third, fourth, and fifth instars and adults but it is **transmitted only by the fourth and fifth instars and adults**

The bacterium has been detected in haemolymph and salivary glands of *D. citri*, and infected **adults retain infectivity throughout their lives**

Young shoots are preferred for feeding and reproduction



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Diaphorina citri



Trioza erytreae



From Silvio Lopes Fundecitrus Brazil

Young shoots have higher rates of transmission



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After 96 hs



% insects that reach phloem **80**

20

% acquisition of *Ca. L. asiaticus* **54**

10

Bonani et al. 2008

Life cycle : egg, 5 nymph instare and adult



ISA-VE



longevity



Rogers and Hall, 2010

The Asian citrus psyllid (*Diaphorina citri*): vector of HLB in America



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45° angle



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D. citri **eggs** are yellow and are often found on the tips of growing shoots. (0.30 x 0.14 mm)

Nymphs are generally yellowish-orange in color and feeds exclusively on new growth. (1st 0.3x0.17mm , 2nd 0.45x0.25mm, 3rd 0.74x0.43mm 4th 1.01x0.7mm 5th 1.6x1.02mm)

Adults are brownish and have brown mottled wings. (3-4 mm)





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D. Citri can fly 5 m and 1,5 km under windy conditions



eggs



nimphs



Adult



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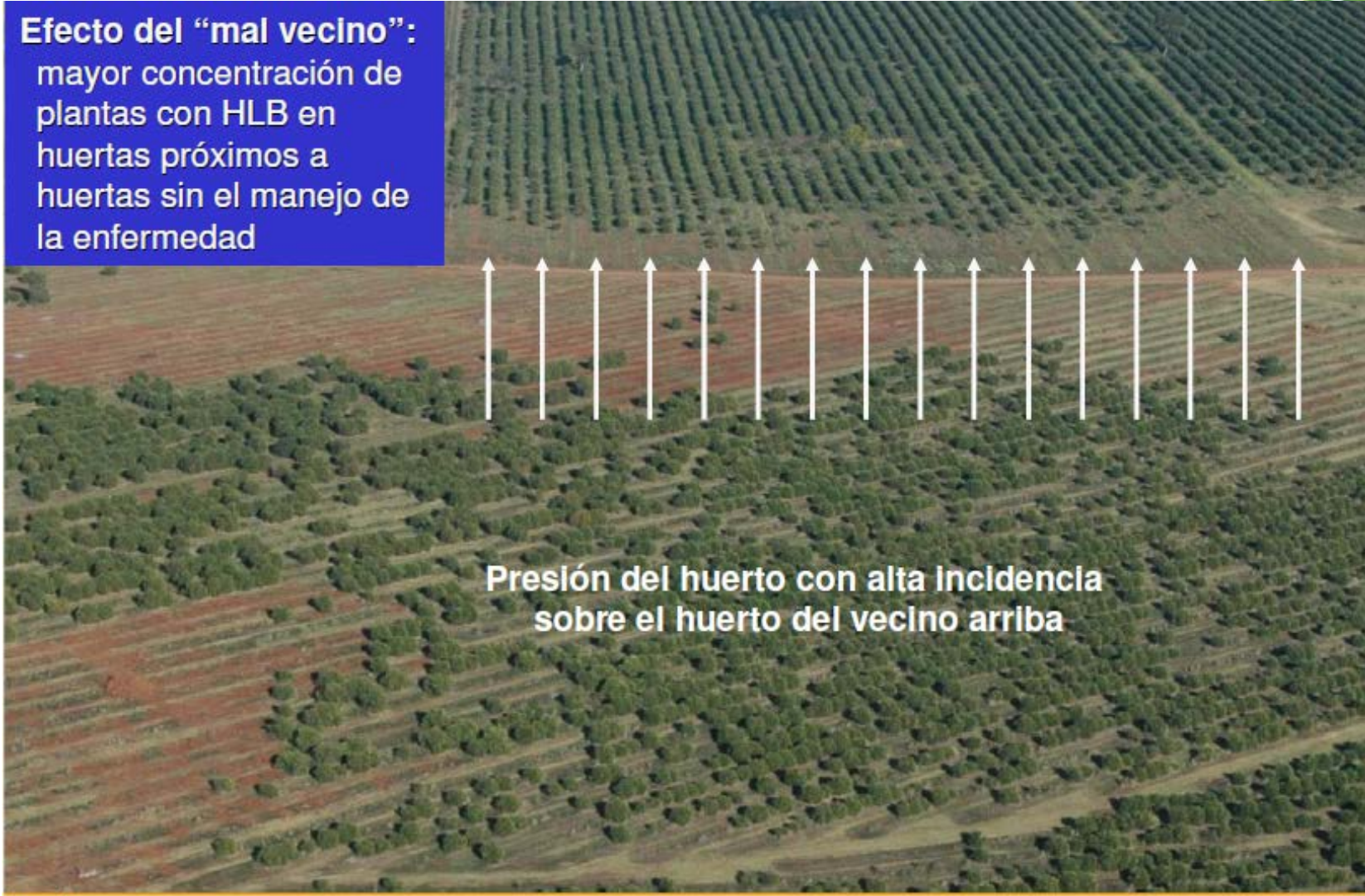




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Bad neighbor effect

Efecto del “mal vecino”:
mayor concentración de plantas con HLB en huertas próximas a huertas sin el manejo de la enfermedad



Presión del huerto con alta incidencia sobre el huerto del vecino arriba

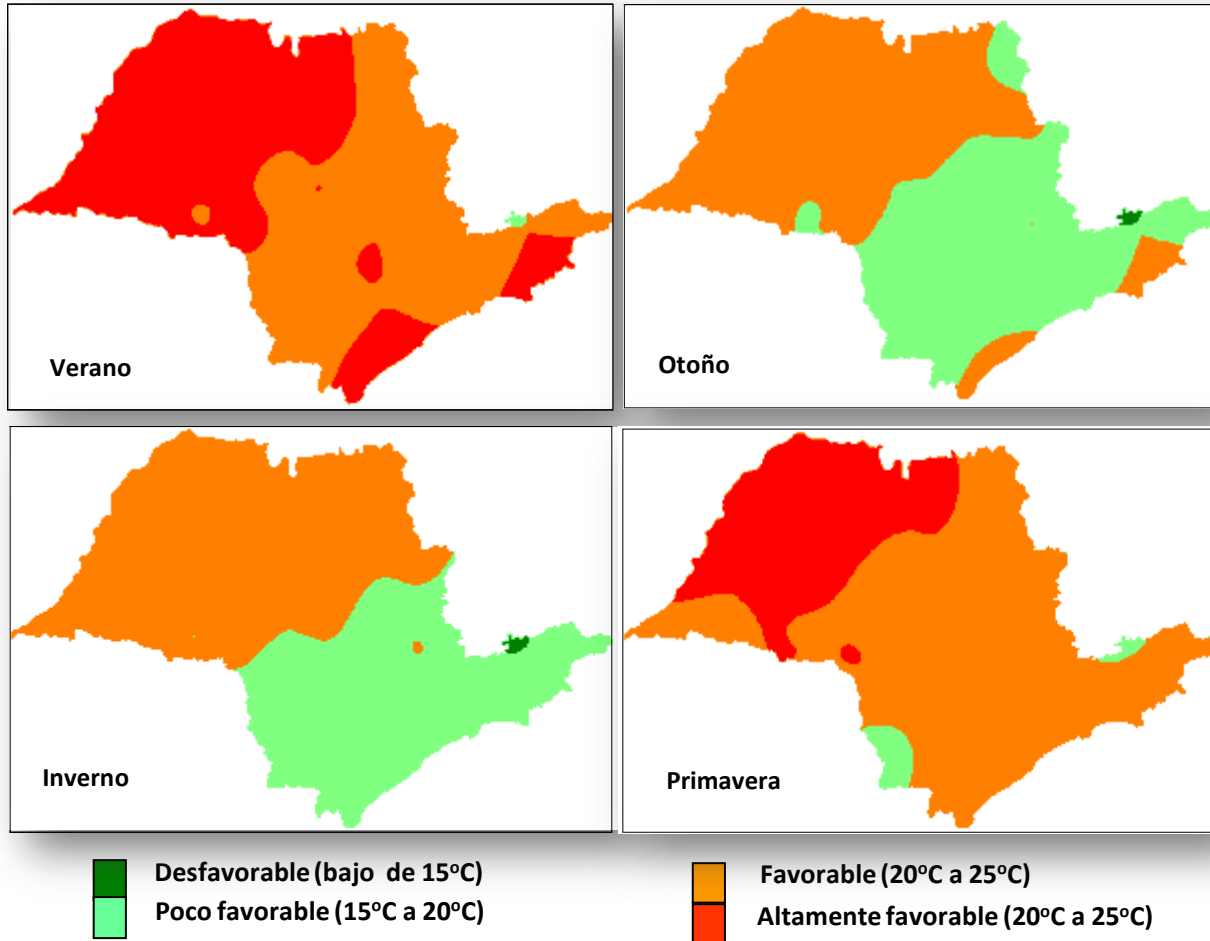
Temperature affects *D. citri*

São Paulo has good conditions for D.c almost all year round



From Silvio Lopes Fundecitrus Brazil

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Morandini et al. 2005



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Agroclimatic Risk Areas for *D. citri* in Argentina

Riesgo agroclimático de las áreas cítricas de Argentina en relación a la abundancia de *Diaphorina citri*

Moschini, R.C.¹; Heit, G.E.²; Conti, H.A.¹; Cazenave, G.¹; Cortese, P.L.²

¹. Instituto de Clima y Agua. CIRN INTA Castelar. Los Reseros y Las Cabañas s/n. B1712WAA. Castelar. Bs. As. Argentina. E-mail: rmoschini@cniia.inta.gov.ar

². Dirección de Vigilancia y Monitoreo. SENASA. Av. Paseo Colón 315. 4º Piso Ofic. 15. Ciudad Autónoma de Buenos Aires. C1063ACD. Bs. As. Argentina

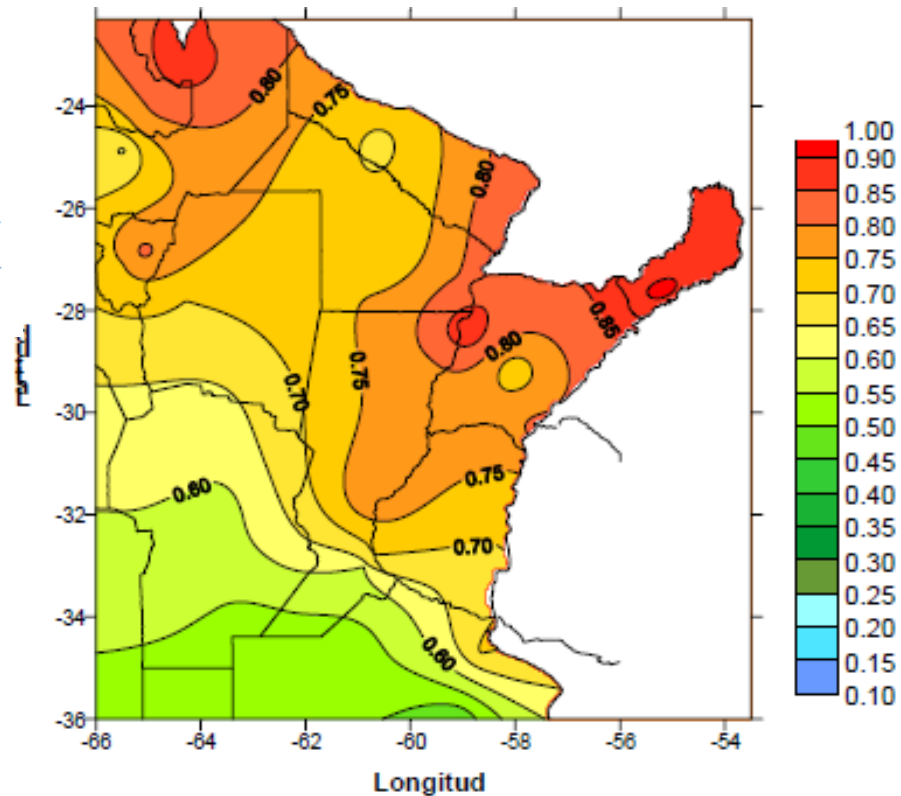


Figura 6. Índice de Riesgo agroclimático 1 (IRA 1) respecto a la presencia y abundancia de poblaciones de *Diaphorina citri* en el centro norte argentino. $IRA1 = (pNGen + pDT + pDTHR + pDSAC)/4$.



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Natural enemies

Tamarixia radiata



***D. citri* parasitized nymphs**



Non-parasitized nymphs



Photos from Bove 2005

27,5 to 80,0% of parasitism has been observed in São Paulo and 56% in Florida

Parra et al. 2006; Qureshi et al. 2009

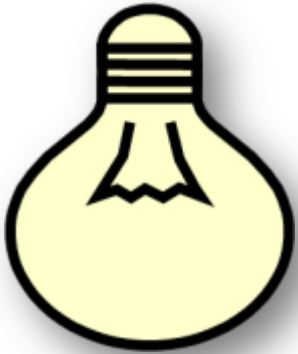
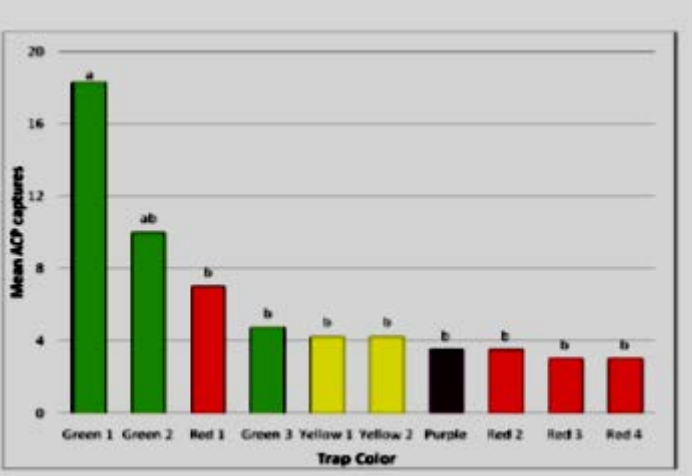
Lights and colors attract adults *D. citri* adults



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Wenninger et al. 2009



Sétamou et al. 2008



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Repellency of volatile substances from *Psidium guajava* spp.



Ishinose et al, 2008



Zaka and Zeng, 2008

•Experimentally confirmed

- Noronha and Bento, 2008

Dimethyl disulfide

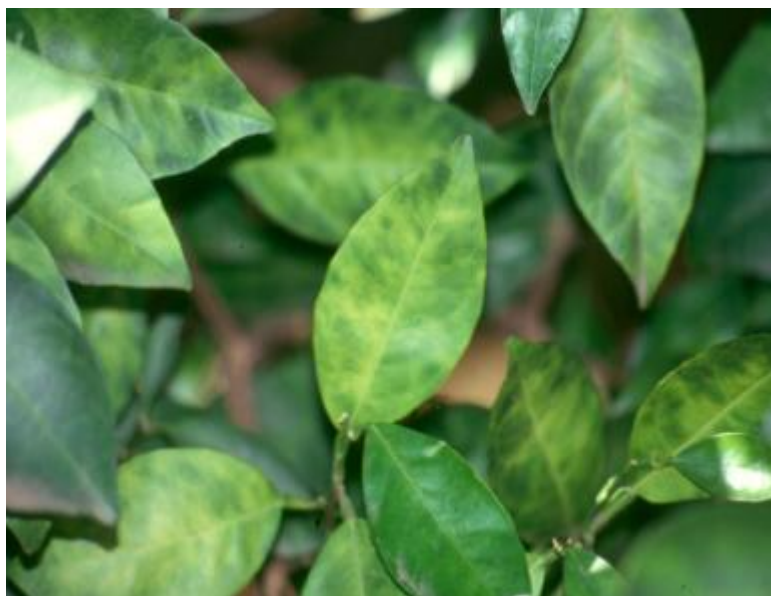
Rouseff et al, 2008

HLB Symptoms



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Very misleading
and tricky disease



Leaf symptoms



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Asymmetric form

Asymmetric chlorosis and irregular discoloration (its possible to confuse with nutrimental deficiencies)

Nervures are chlorotic and thicker, sometimes with corking aspects.

Yellow mottling is the most characteristic symptom



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Florida



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China



J. M. Bové

São Paulo



South Africa



Yellow mottling leaves is the most useful symptom to identify diseased trees in the orchard



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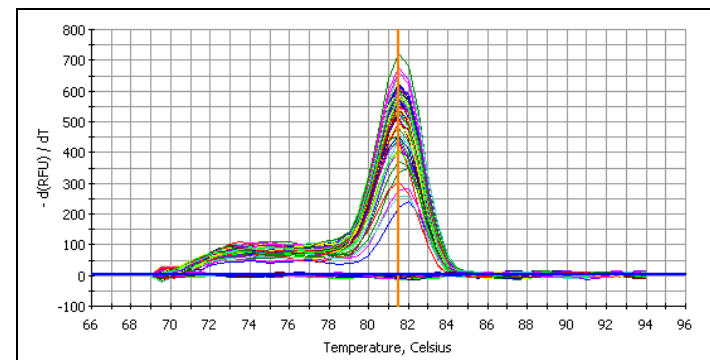
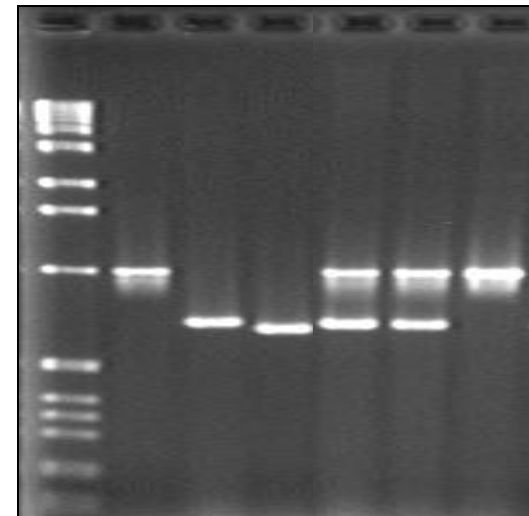
The mottling symptom is not always easily identified



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Confirmation is only possible by means of PCR



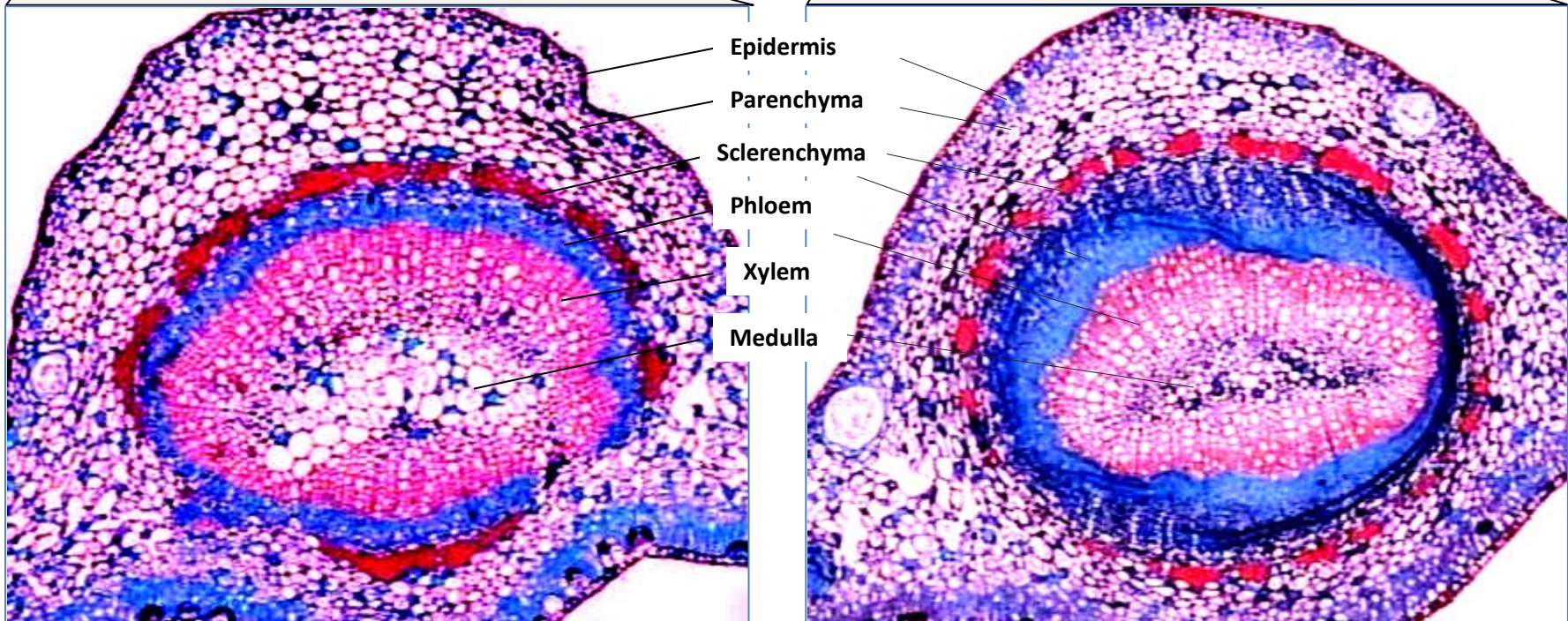
Li et al. 2006

Teixeira et al. 2008



E

The mottling is associated with high phloem cells proliferation



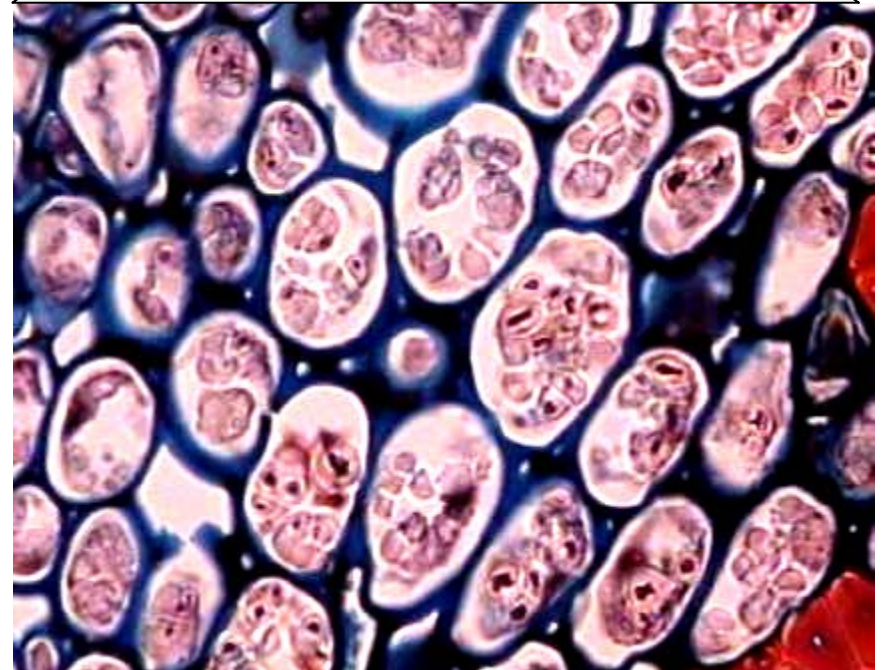
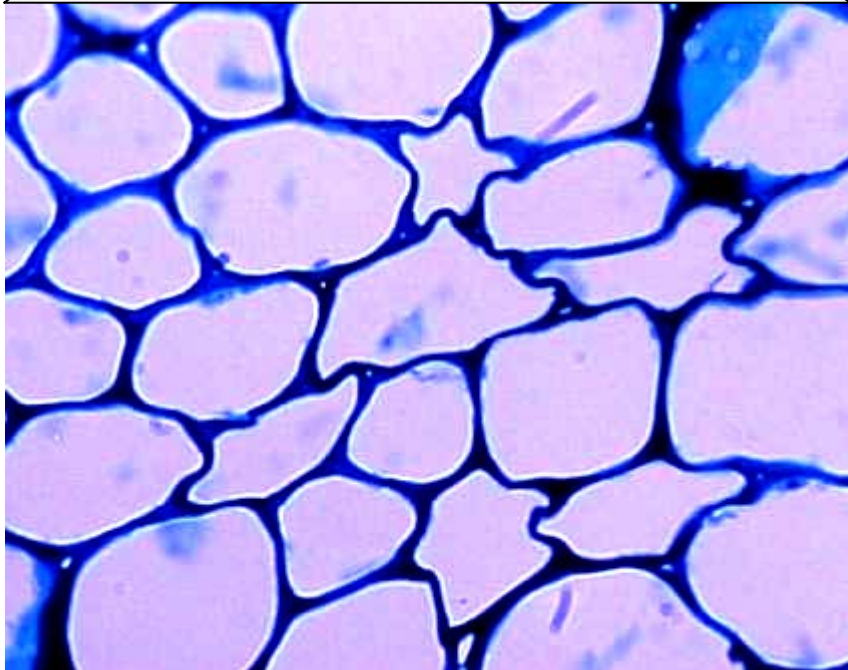
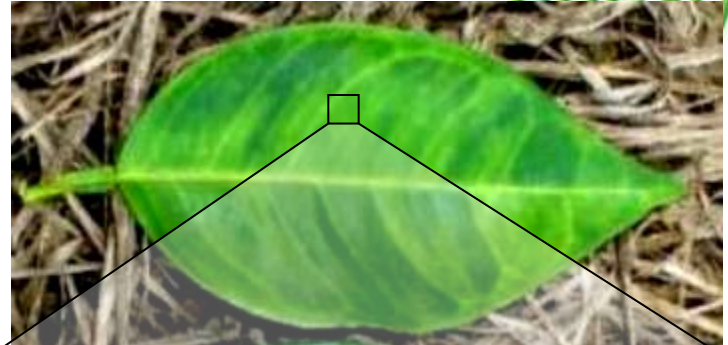
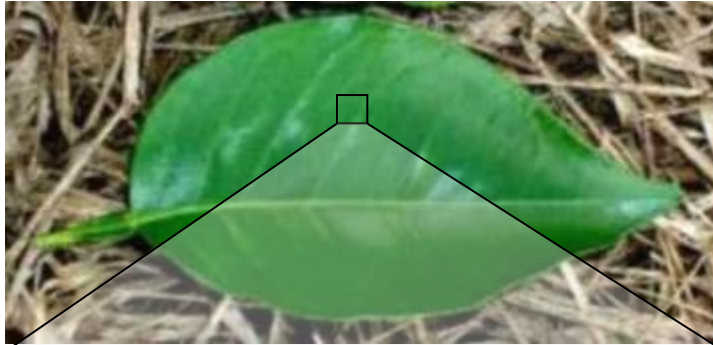
Schneider et al., 1968; Heredia et al, 2006

From Silvio Lopes Fundecitrus Brazil



ECOLOGY

High level of starch in parenquimatic cells



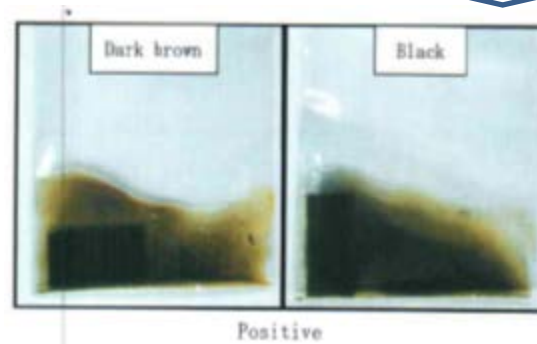
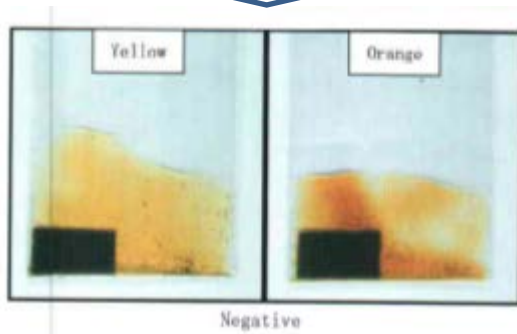


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Iodine test



Takushi et al. 2007



Beattie, 2007

•76% correspondence with PCR results.

(Chamberlain and Irej 2008)





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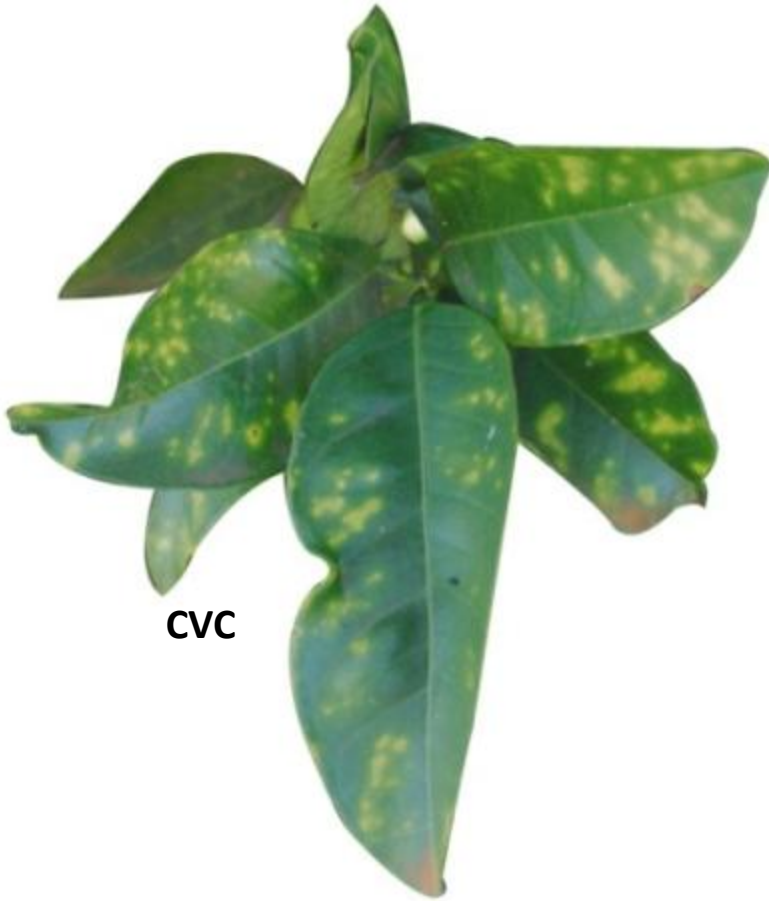
nutritional deficiencies



Zinc (Zn)



Manganeso (Mn)



CVC



Gummosis



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Fruit symptoms

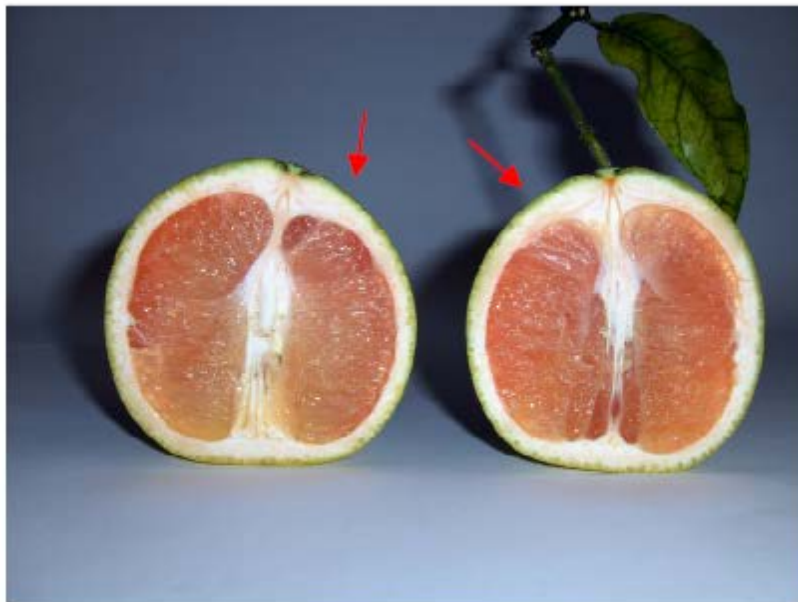
- Deformation (asymmetric)
- Smaller fruit
- Abort seeds
- Inversion of maturation color
- Premature drop







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Lopsided fruit with a curved central core



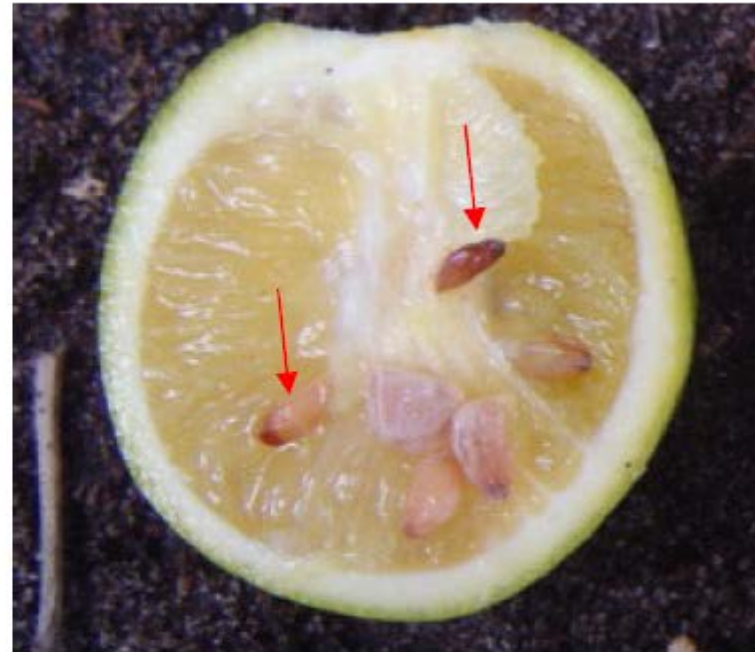
Yellow stain beneath the peduncle (stem) on a cut fruit.



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Irregular coloration



Aborted seeds

Symptoms evolution



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The symptoms expression takes 6 months to 2 years



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Very quick evolution in young plants (1 to 3 months)



Citrus orchard destroyed by Greening (J.M.Bove)



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The quick spread of the bacteria throughout the plant makes the cure impossible



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Example Test

216 trees were pruned

The symptoms reappeared in

58,3 %



376 trees were pruned

The symptoms reappeared in

62,5%



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Chronically infected trees display extensive twig and limb dieback, tend to drop fruit prematurely, and are sparsely foliated with small leaves that point upward

Main alternative host: *Murraya paniculata*



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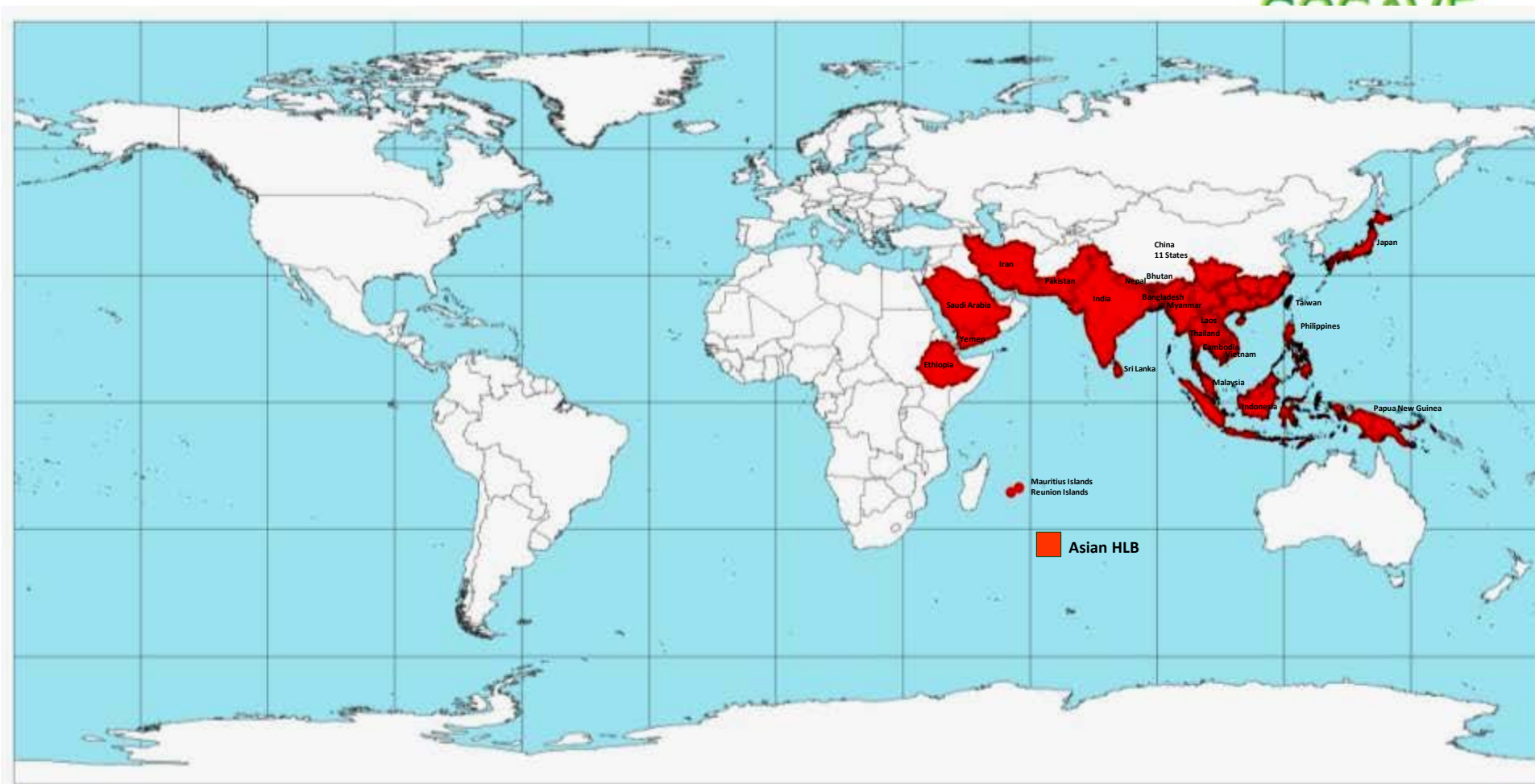
It is not known when it was first reported





ICCCRP

Today Asiatic HLB is present in Asia, Arabic peninsula and some African countries

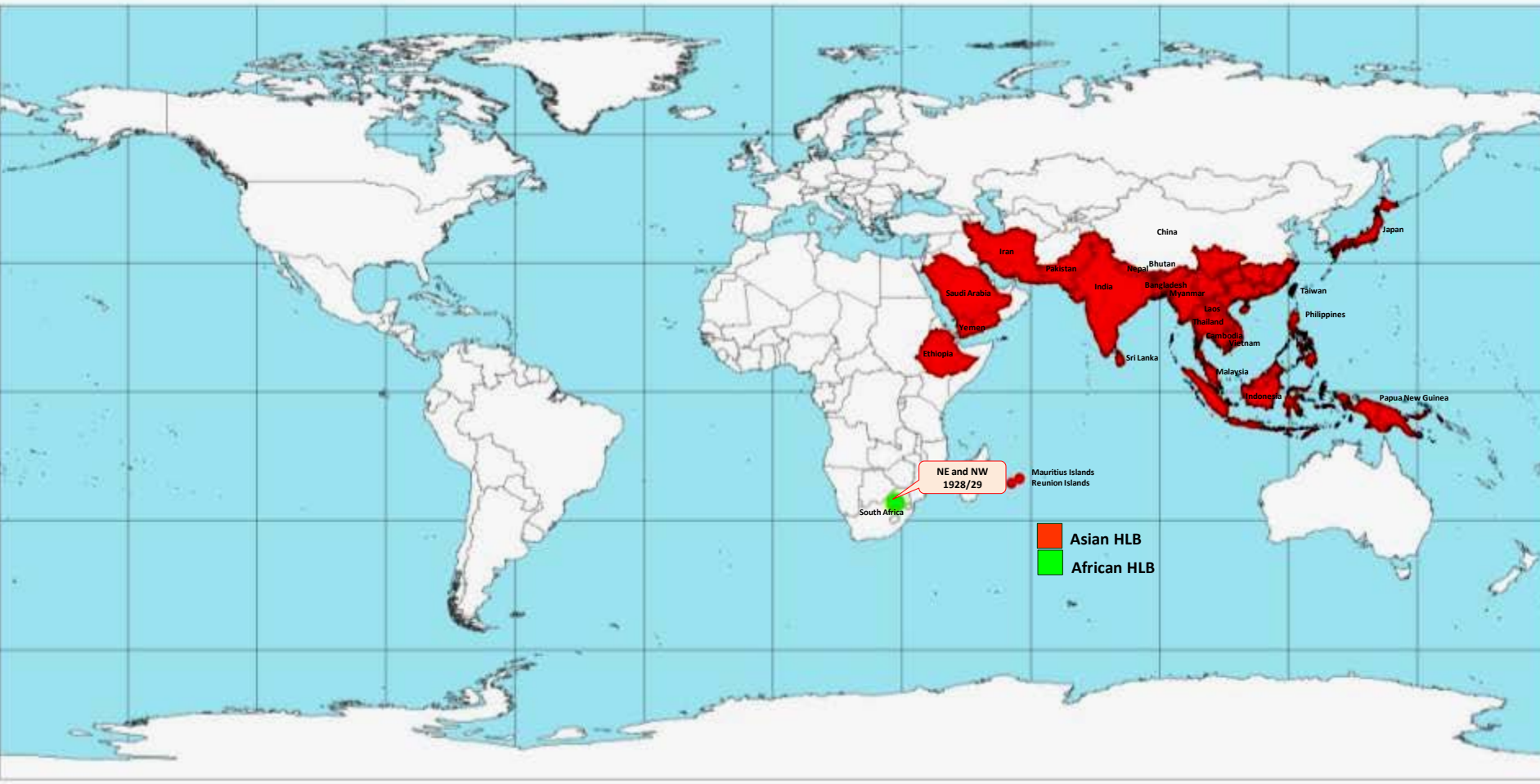


From Silvio Lopes Fundecitrus Brazil

1928: another form of Liberibacter (*Liberibacter africanus*) (under greening name) was informed in the north of South Africa



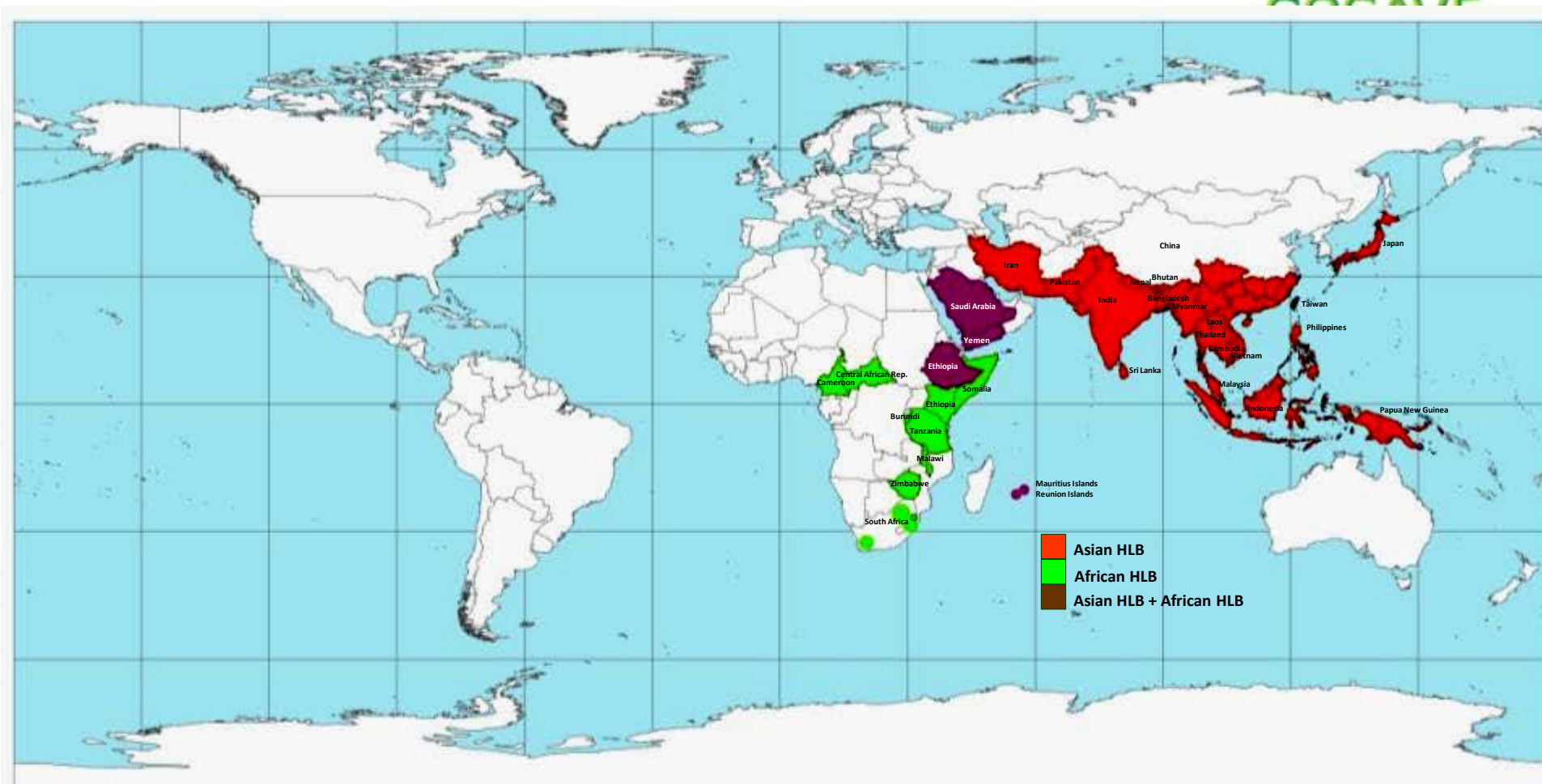
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ICISA

Today Greening is present in some parts of Africa and Saudi Arabia Saudita and Yemen

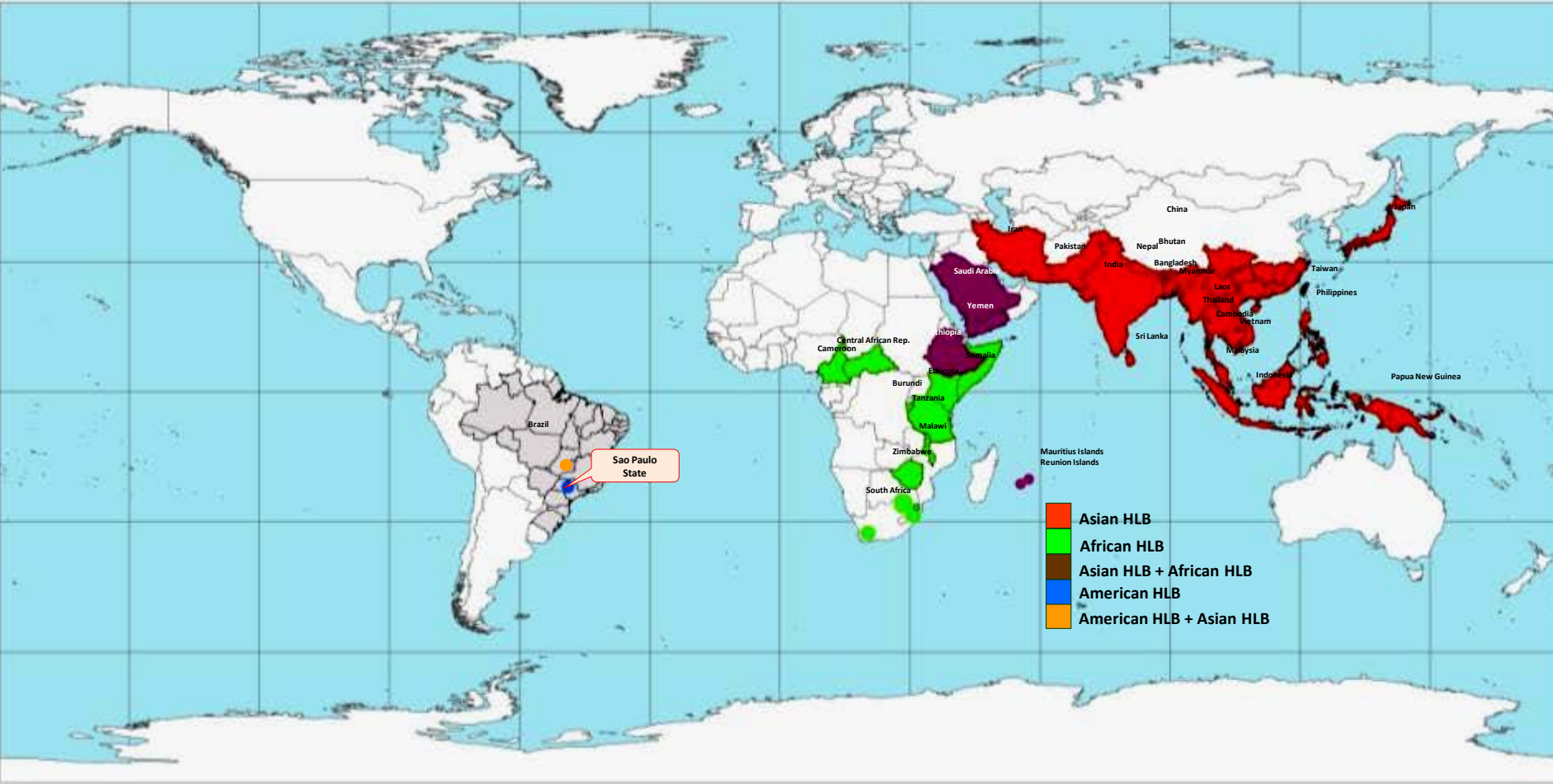


From Silvio Lopes Fundecitrus Brazil

2004: A third Liberibacter species (*Liberibacter americanus*) was reported in São Paulo Brazil



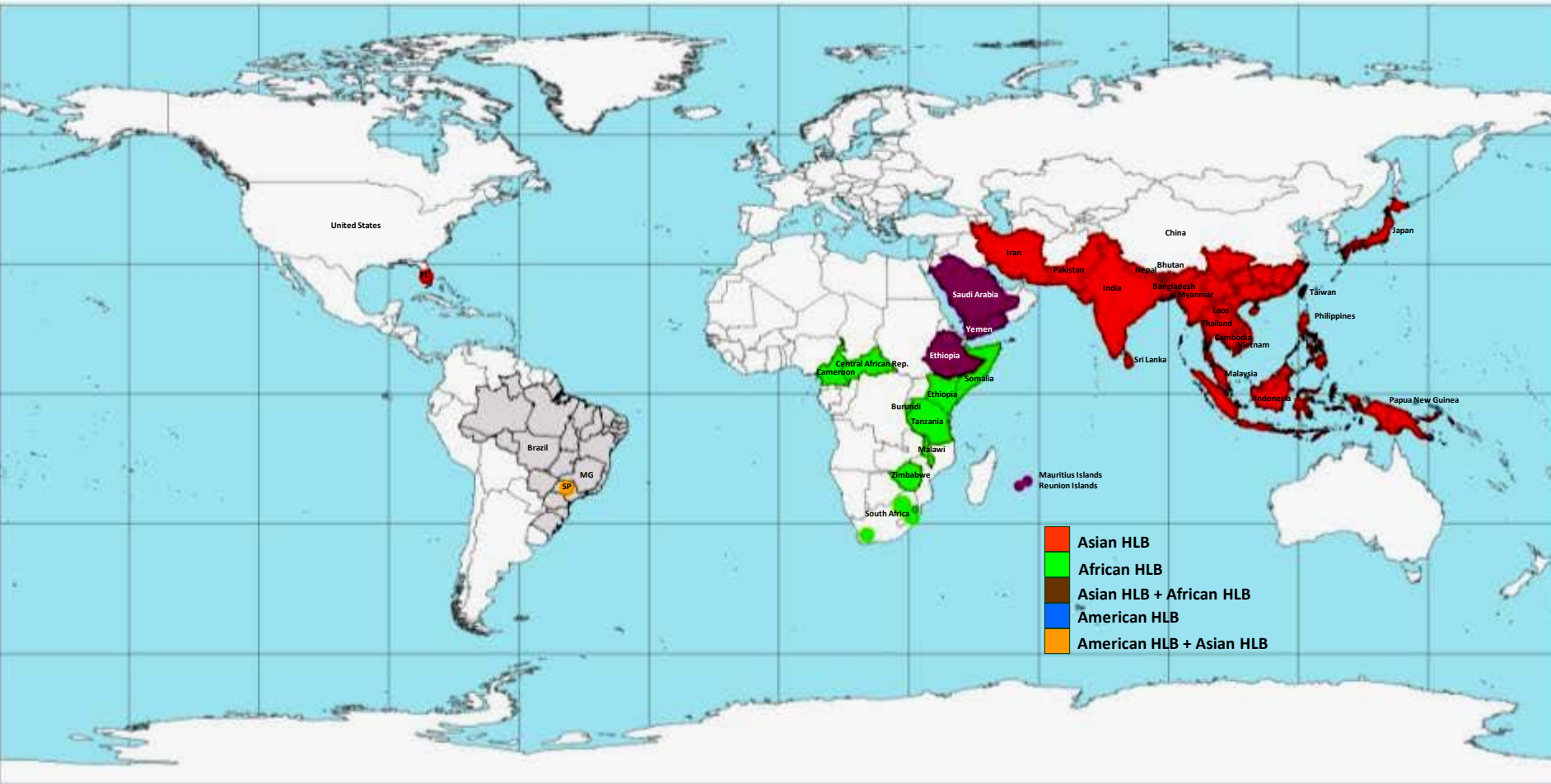
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2005 Asiatic HLB was reported in Florida USA



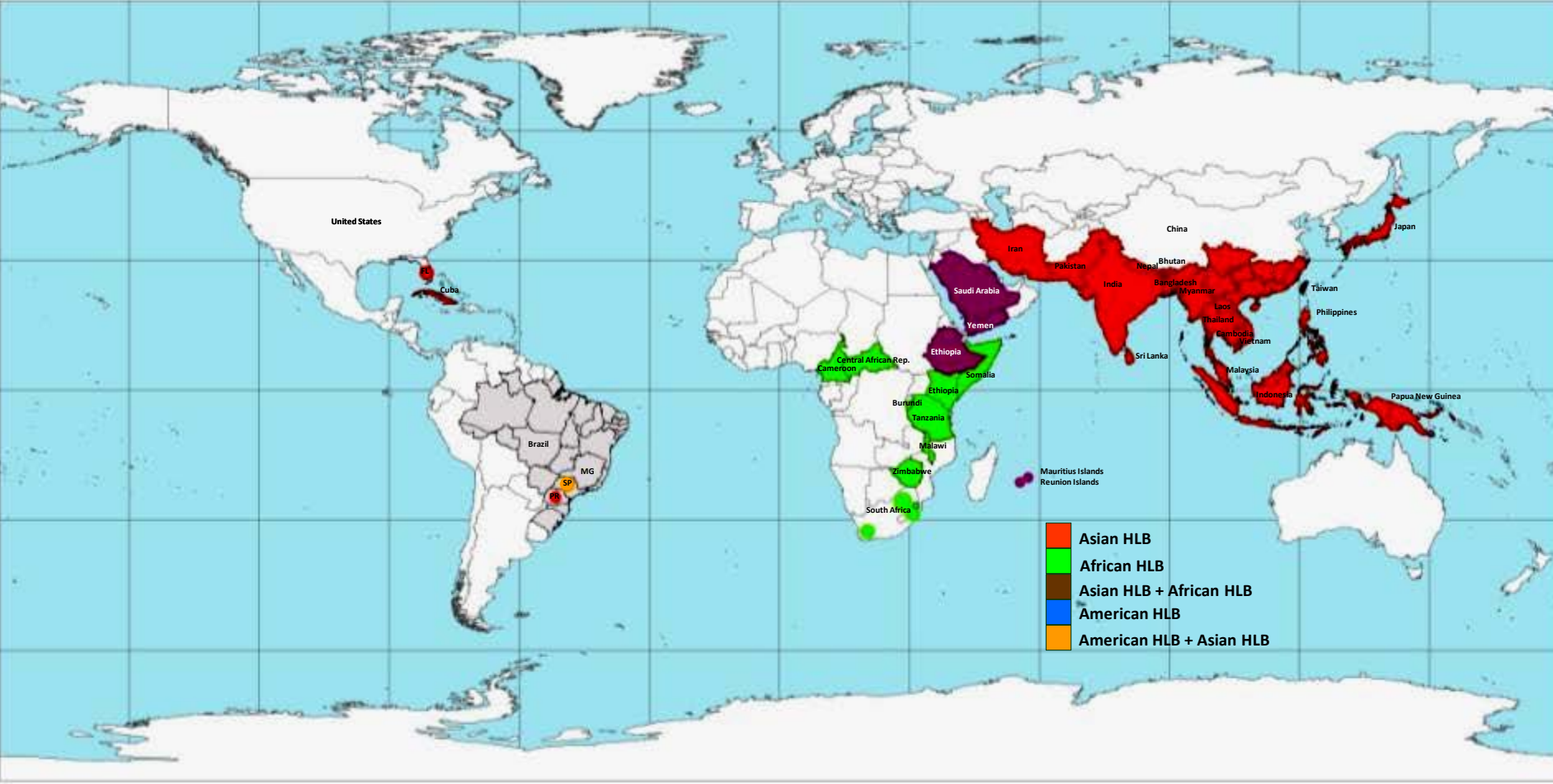
CCOAV



2006: Asiatic HLB was found in Cuba and Paraná do Brasil State (Brazil)



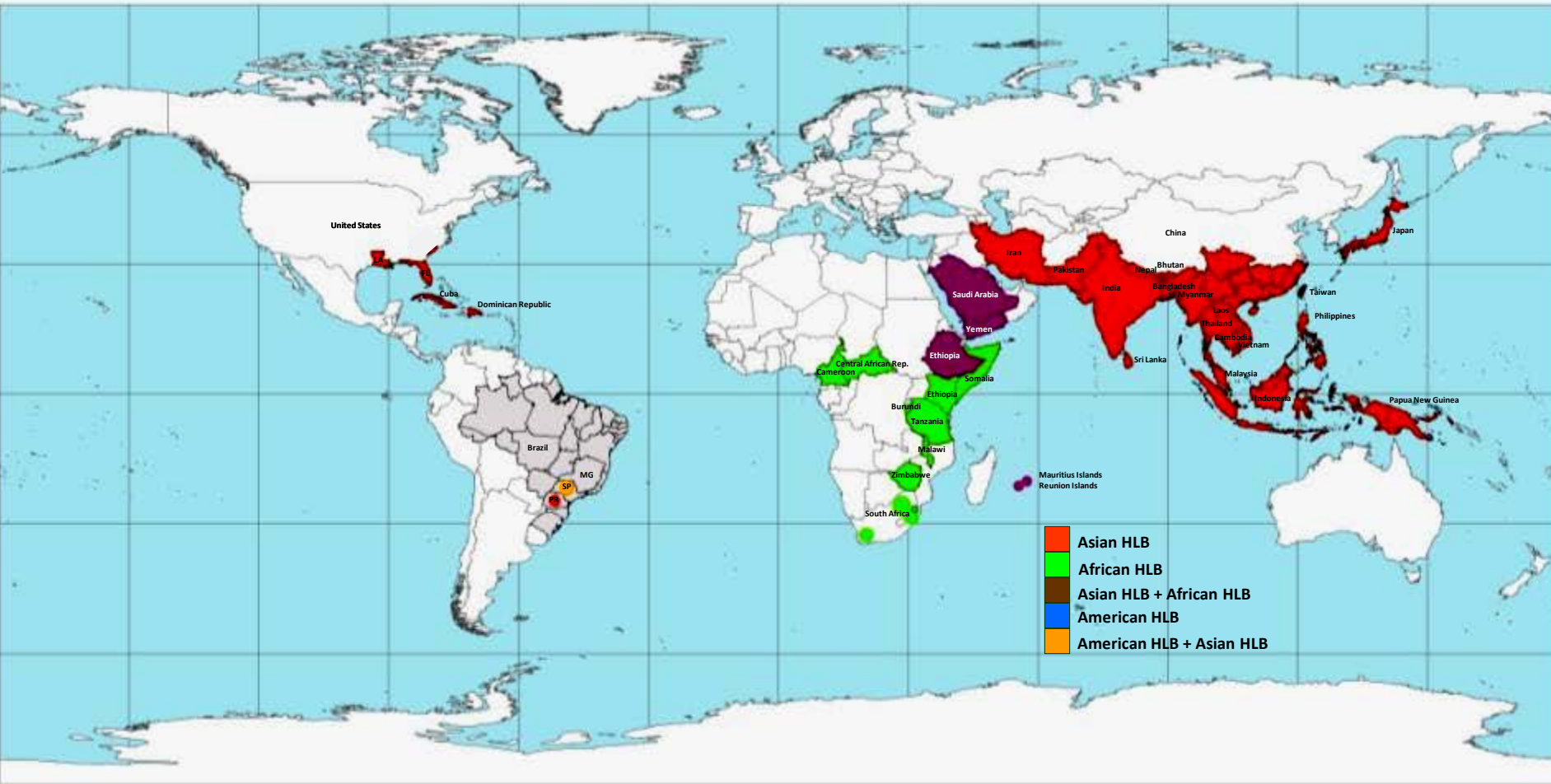
ICIPE



2008: HLB Asiatic was reported in República Dominicana and Luisiana (USA)

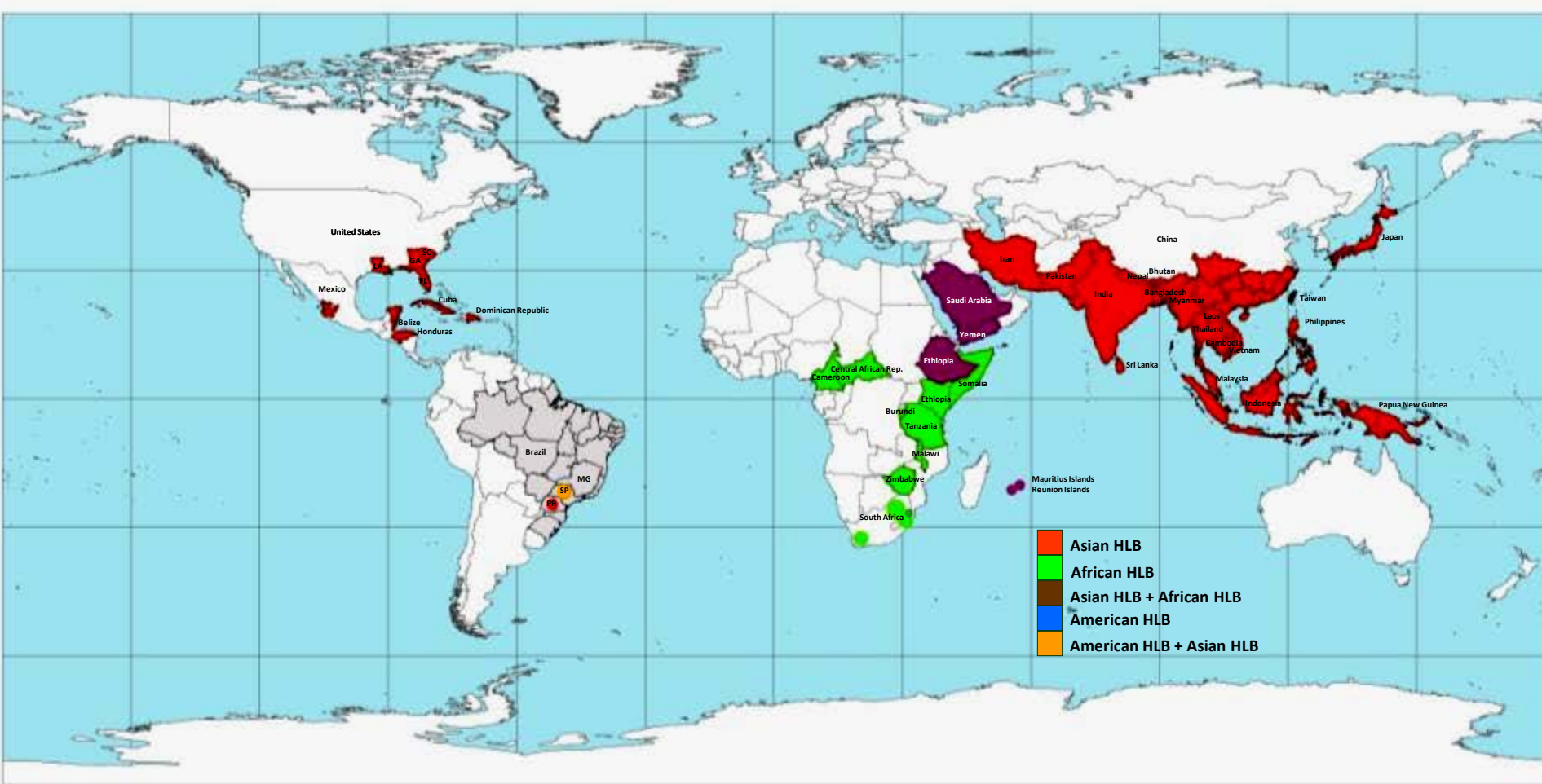


IPPC





2009 : Asiaticus was reported in Honduras and Belice, Georgia and South Carolina (USA) and in Yucatán, Jalisco, Nayarit and Quintana Roo (Mexico)

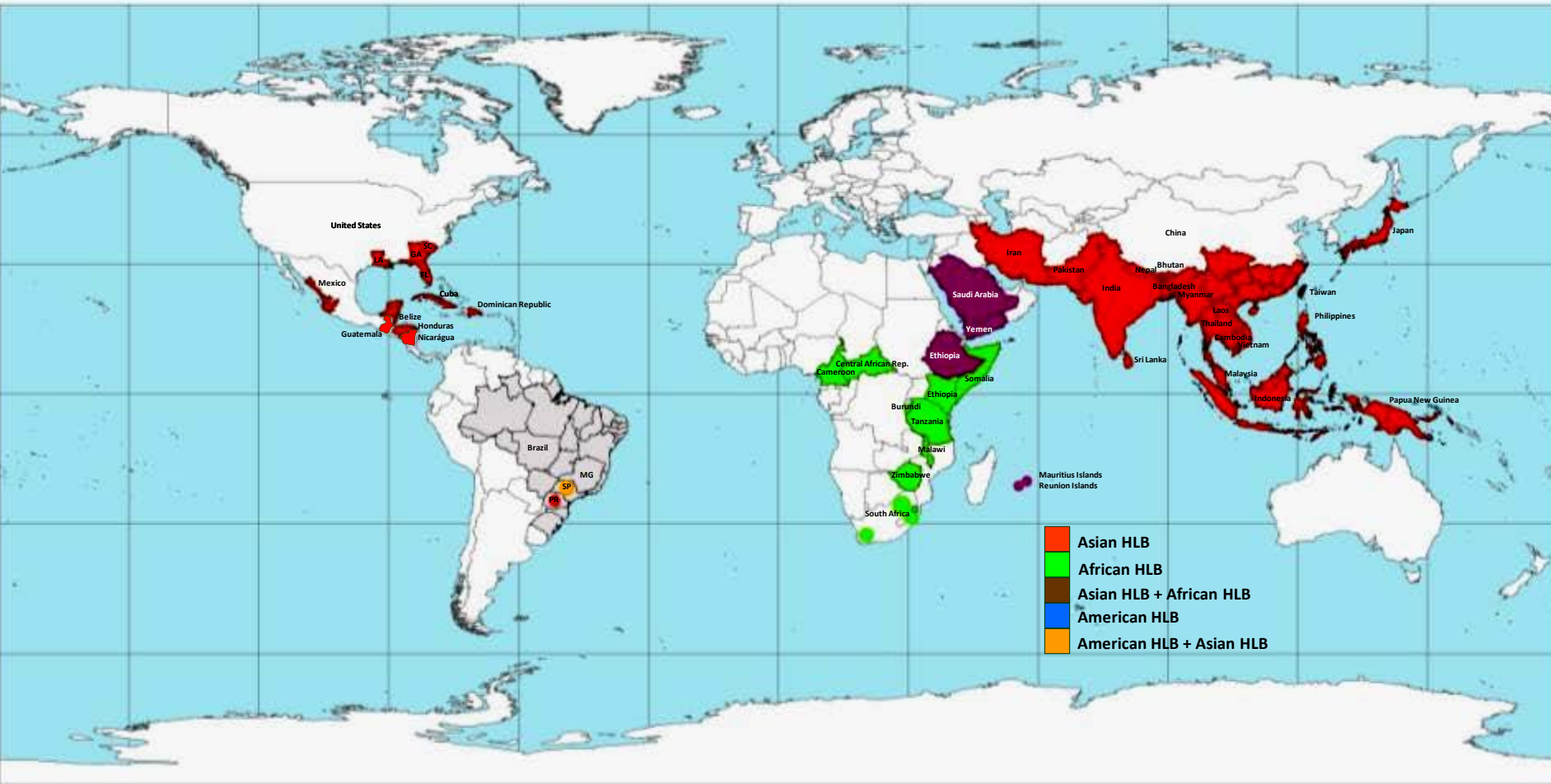


From Silvio Lopes Fundecitrus Brazil

2010: Asiatic in Guatemala, Nicaragua, and 3 more States of México (Campeche, Colima and Sinaloa)



CCOAV

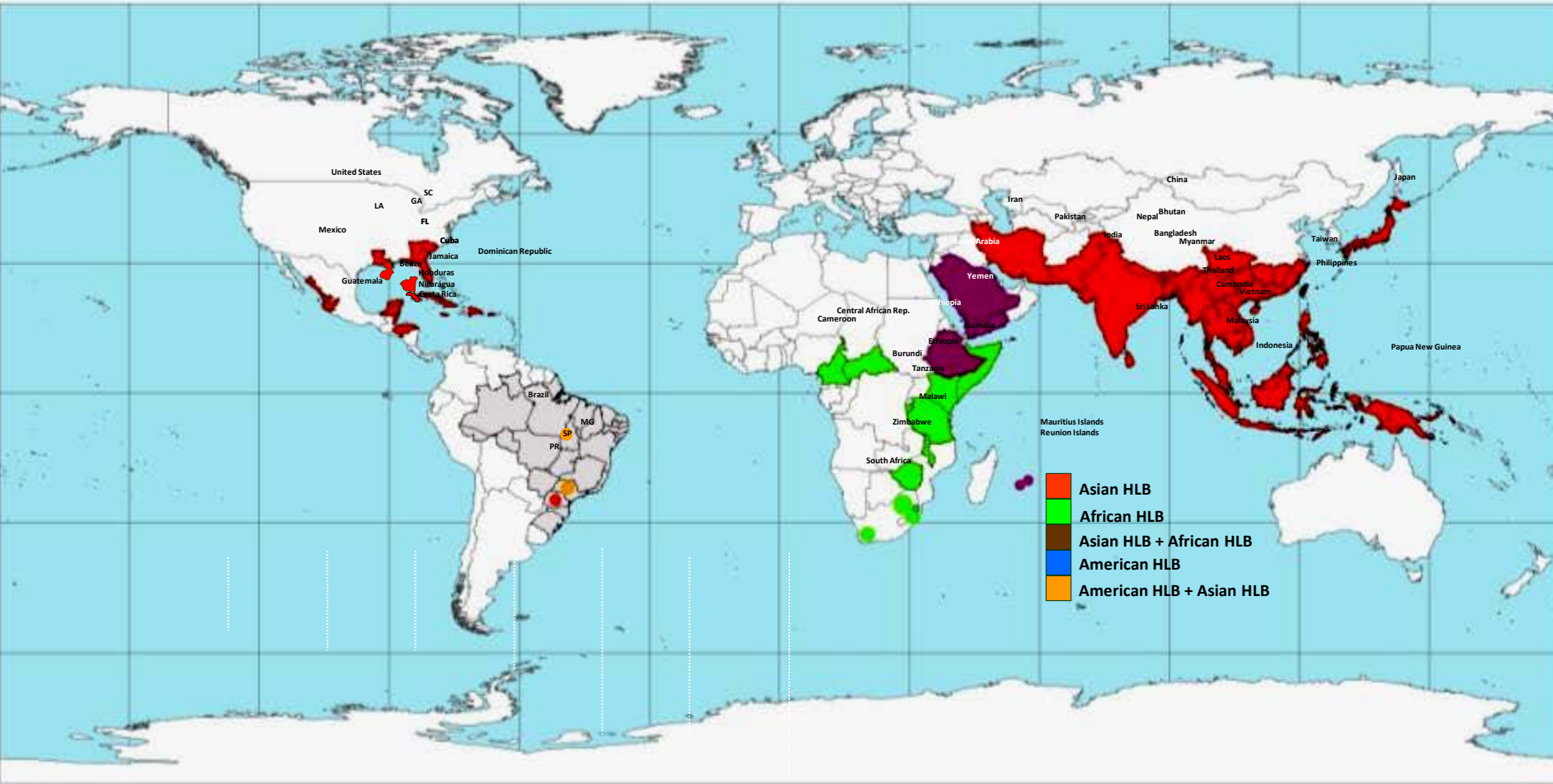


From Silvio Lopes Fundecitrus Brazil

2011: Asiatic in Costa Rica and Jamaica



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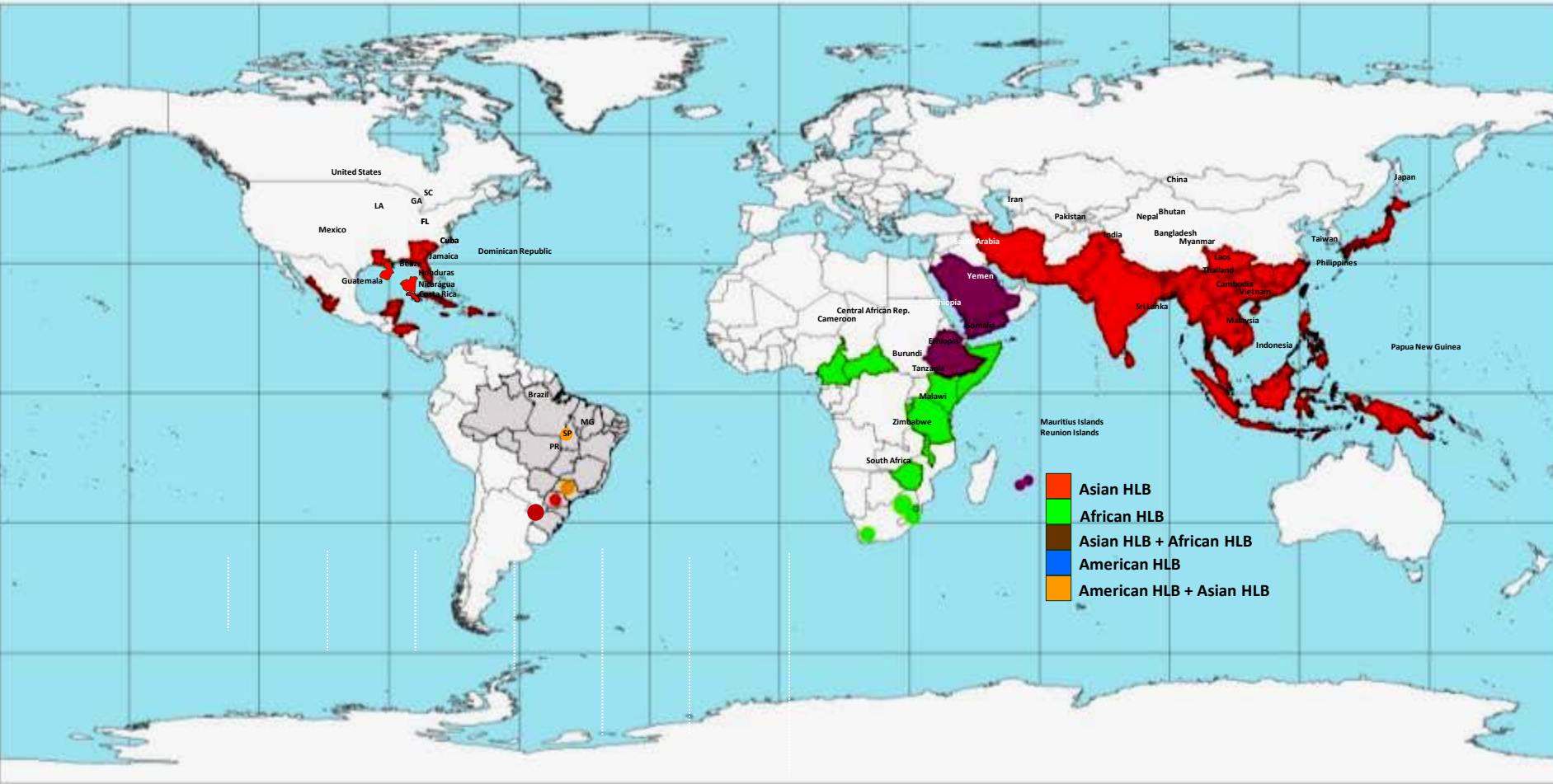


From Silvio Lopes Fundecitrus Brazil

2012: Asiatic was found in Argentine borders with Brazil (Misiones Province) now under eradication



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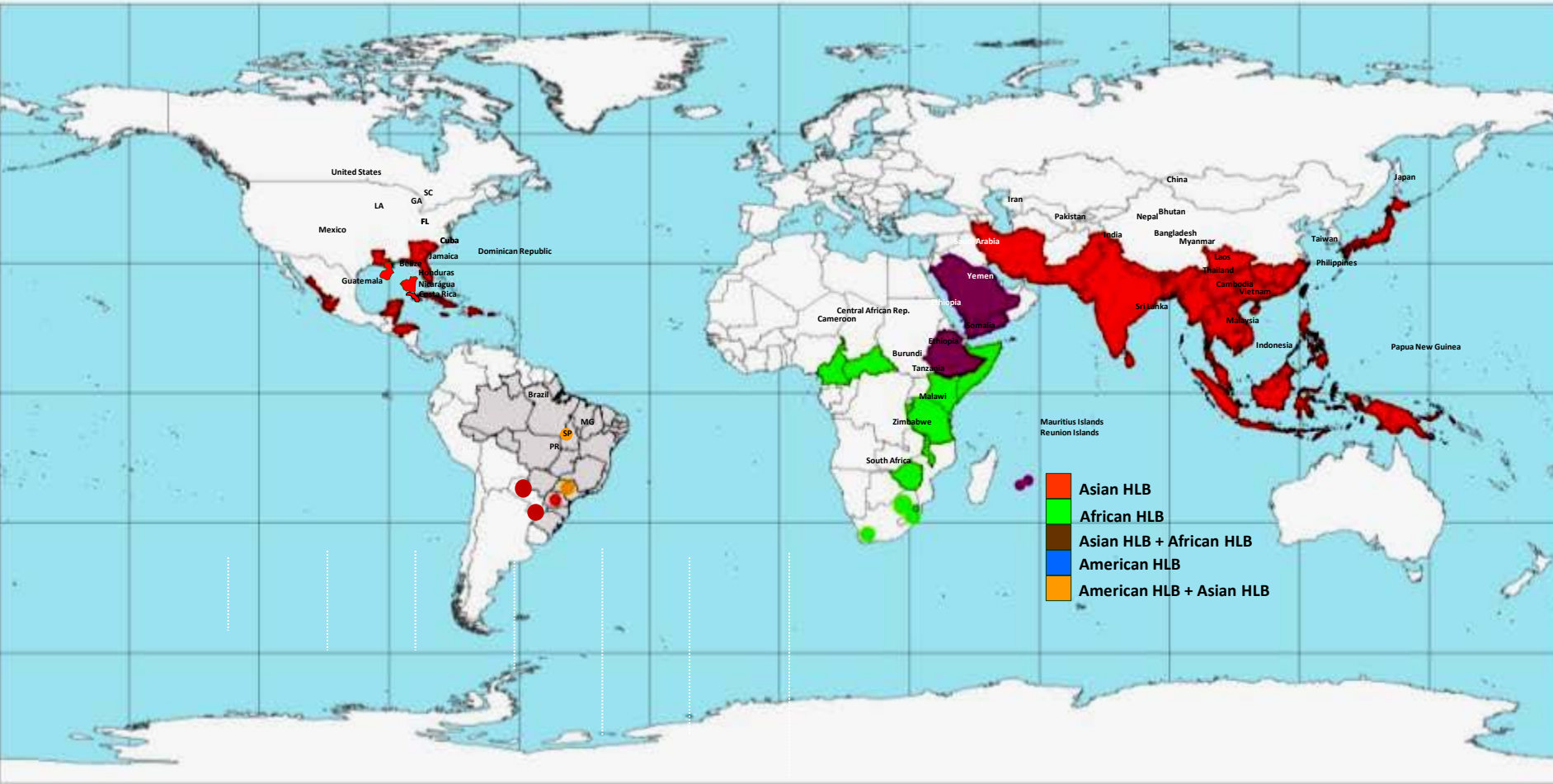


From Silvio Lopes Fundecitrus Brazil

2013: Asiatic was reported by Paraguay in 8 departments



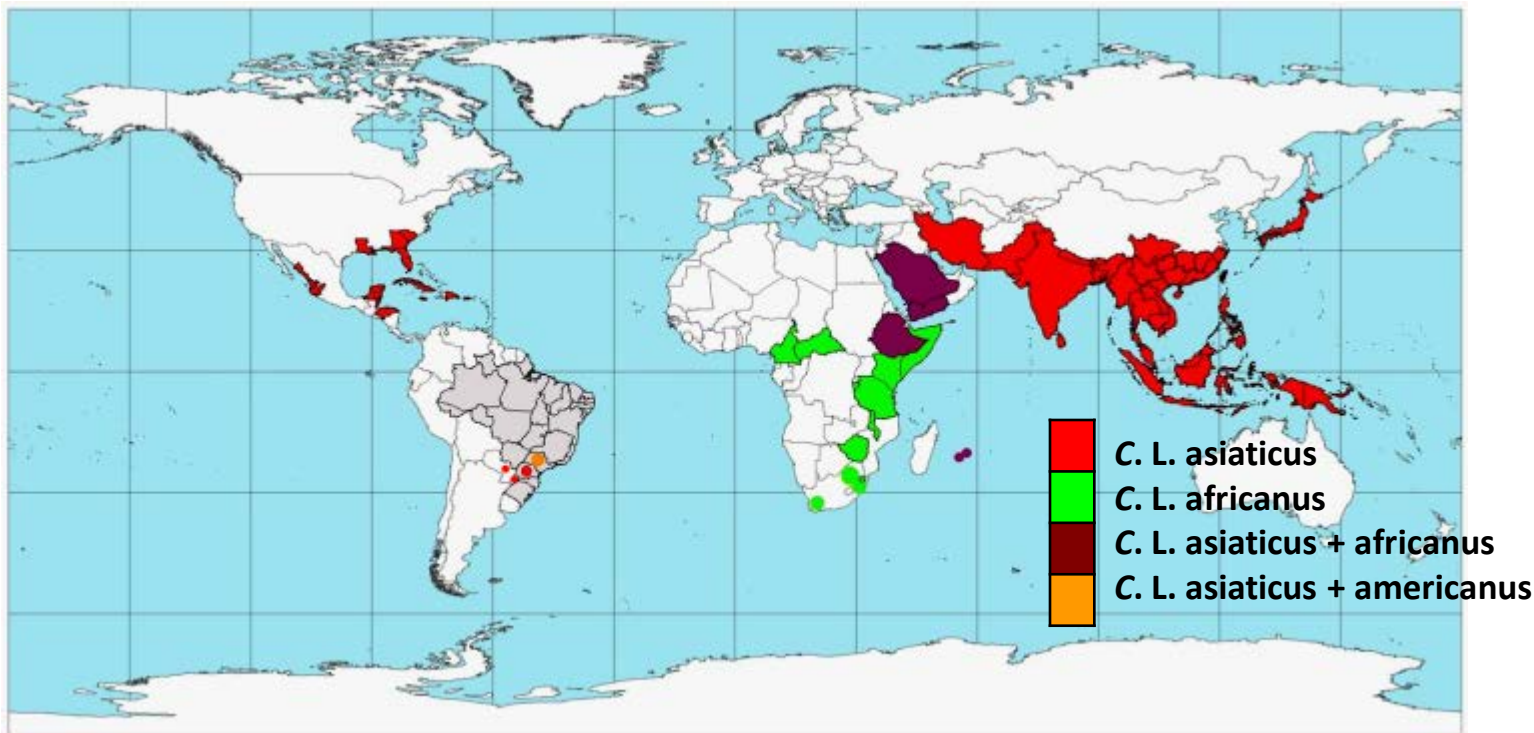
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- First detection in China, 1890 (asiatic pv)
- 1937, it was detected in South África (african pv) and in other countries of Asia and Africa
- March 2004 it was detected in Araraquara, Brazil (Asiatic and American)
- September 2005, it was reported in Florida, USA and after in other countries of Central America
- June 2012 it was detected in Misiones, Argentina. Area under Contingency plan
- March 2013 it was detected in many counties of Paraguay



Asia: China, Irán, Pakistán, India, Nepal, Bhutan, Bangladesh, Myanmar, Laos, Tailandia, Cambodia, Vietnam, Filipinas, Japón, Nueva Guinea, Malasia, Indonesia, Sri Lanka.

África: Arabia Saudita, Yemen, Etiopia, Camerún, Rep. África Central, Somalia, Burundi, Tanzania, Malawi, Zimbabwe, Sudáfrica, Islas Mauricio, Islas Reunión.

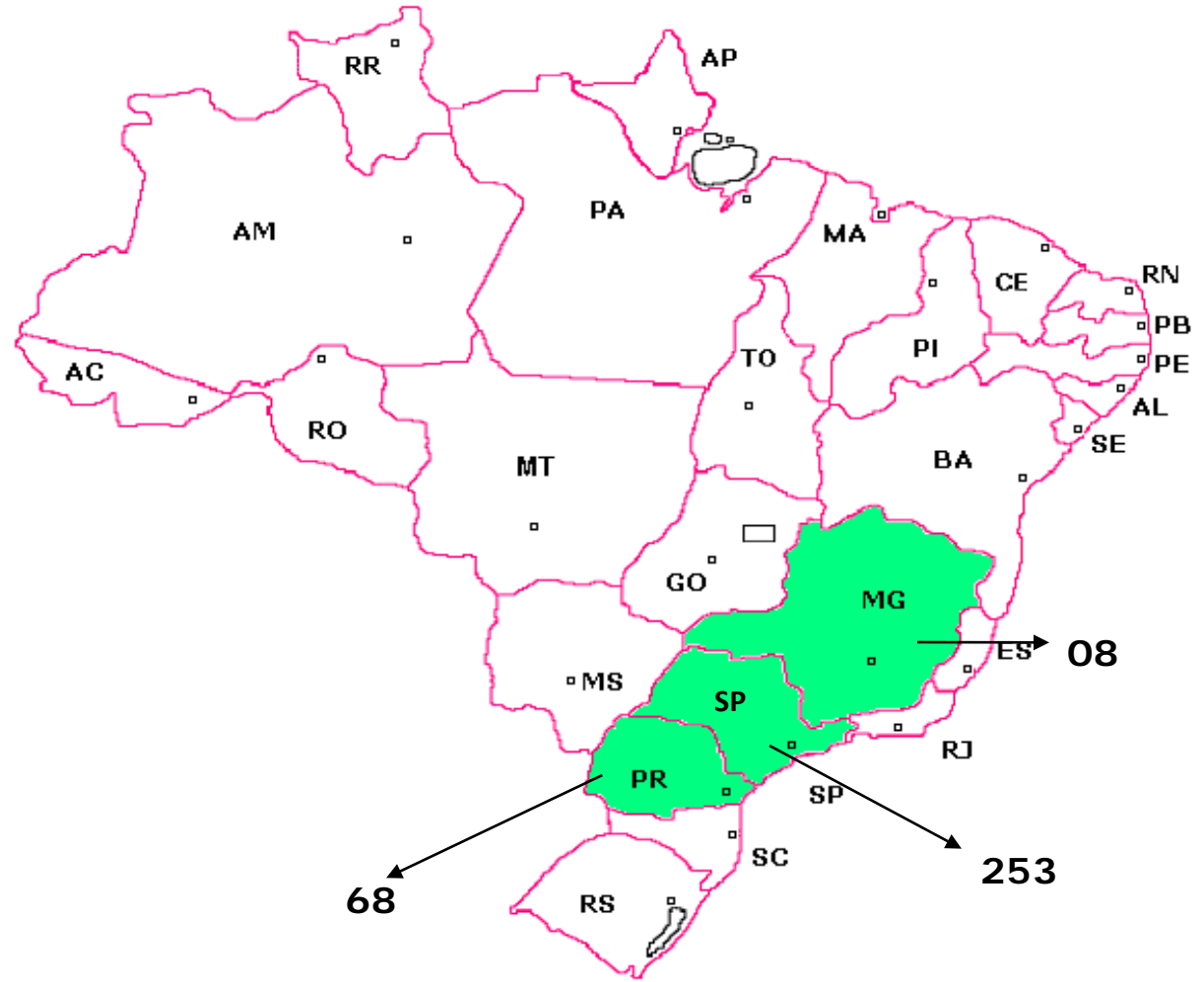
North America: Estados Unidos (2005).

America Central and Caribe: Méjico(2009), Cuba (2007), Jamaica, República Dominicana (2008), Belize (2009), Honduras, Costa Rica, Nicaragua, Guatemala, Jamaica.

Sudamérica: Brasil (2004), Paraguay (2013), Argentina (2012).

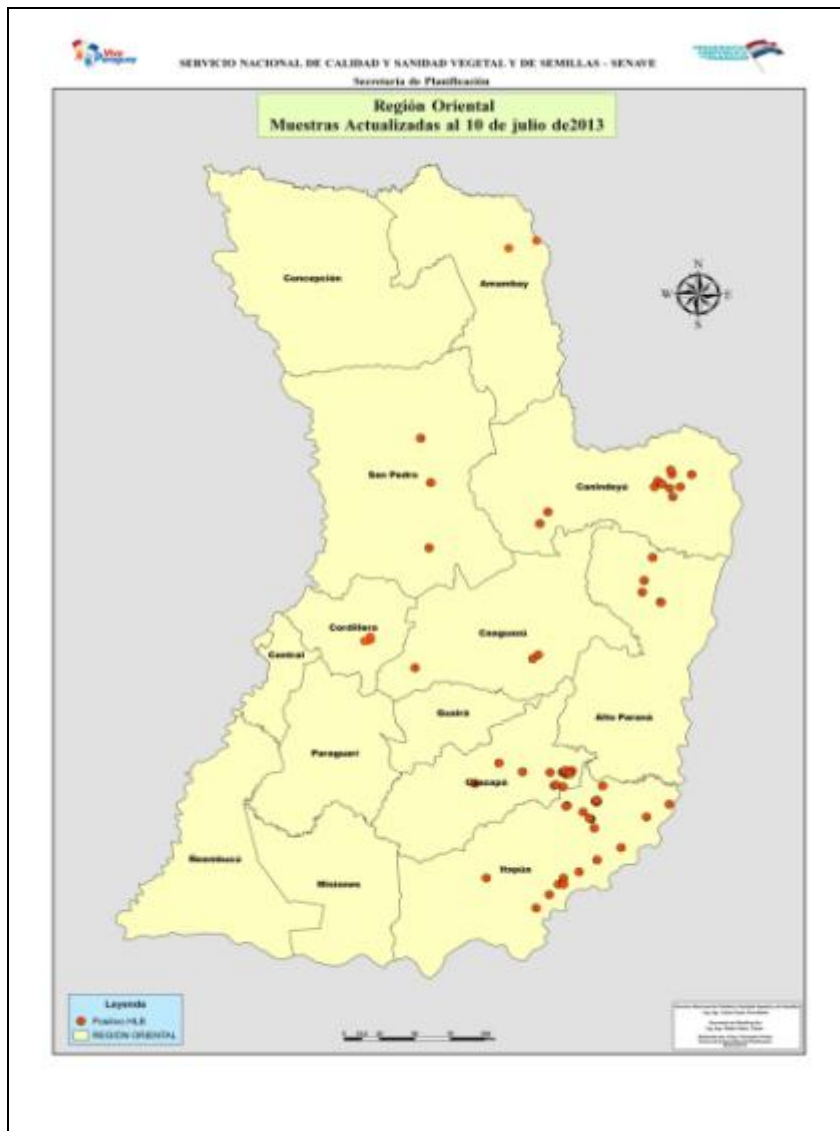


HLB in Brazil





COSAVE



HLB in Paraguay

CITRICOS CON HLB ERRADICADOS CAMPAÑA 2012-2013

DIRECCION NACIONAL DE PROTECCION VEGETAL

CENTRO REGIONAL CORRIENTES-MISIONES

Departamento General Belgrano
Provincia de Misiones

REFERENCIAS

Positivos

• Año 2012

• Año 2013

• Localidades

— Limite departamental

Rutas

— CONSOLIDADO

— DE TIERRA

— PAVIMENTADO

— PAVIMENTO EN CONSTRUCC

— Cursos agua

— Grilla 1000 ha

— Grilla 100 ha

— Limite departamental

— Catastro

— Parque Nacional

— Cultivos citricos



Kilómetros

1:101736



COSAVE

HLB in Argentina



Economic importance of citrus in Cosave Region

Country	Tons	Hectares
Argentina	2.500.000	140.000
Brazil	20.000.000	900.000
Chile	153.000	18.500
Paraguay	219.000	18.000
Uruguay	270.000	16.179



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HLB in COSAVE Region

Country	Confirmed presence of <i>D. citri</i>	Confirmed presence of disease
Argentina	★	★
Bolivia		
Brazil		★
Chile	★	
Paraguay	★	★
Uruguay	★	



National Programs in each country

Country	
Argentina	Yes
Brazil	Yes
Chile	Yes
Paraguay	Yes
Uruguay	Yes
Bolivia	No





General Goal

- Prevent the spread of HLB through performing regionally coordinated national action

Specific Goals

- Prevent HLB entry to countries that are currently free, and limit its spread to where HLB is present.
- Prevent the entry and spread of *Diaphorina citri* (Asian citrus psyllid) to countries where it is not present today
- Prevent *Trioza erytreae* entry to COSAVE Region (African citrus psyllid)



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Regional Activities (Joint actions)



COMMUNICATION



TRAINING



DIAGNOSTIC ASSISTANCE



RESEARCH



PLANT PROPAGATION
INTRODUCTION



COSAVE

COMMUNICATION ACTIVITIES

All National information on COSAVE web site (National Program documents, legislation, surveillance information, etc)

Regional quick alert system

Web map with regional distribution of the disease and *Diaphorina citri* in COSAVE Region

(fecha de la presentación)

JOINT TRAINING



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Set up a laboratory net (22 today)





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GUIDELINES FOR THE SAFE INTRODUCTION OF MATERIAL PROPAGATION

Agree on such guidelines among countries of COSAVE



Frontier Problems



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- High risk of illegal introduction by tourists or in transit passengers
- Social and cultural problem
- Difficulty to control



Argentine HLB Prevention Program



COSAVE

N°26888 National Law



National Service for Agrifood Health and Quality

Program specific goals:



- Regulatory framework
- Surveillance systems for early detection of HLB.
- Diaphorina citri control
- Mandatory official control over HLB for the whole citrus plant production
- Design and prepare contingency plans
- Specific researches
- Training, awareness and communication activities

Argentine Surveillance system



- Monitoring and sampling of *Diaphorina citri*.
- Monitoring commercial plots and urban plants , sampling suspicious symptoms
- Trapping net in free areas of *Diaphorina citri* for early detection.
- Online system to collect data and laboratories results.
- National Laboratories Net (PCR real time analysis)

Monitoring sites

Its was determined by



reas

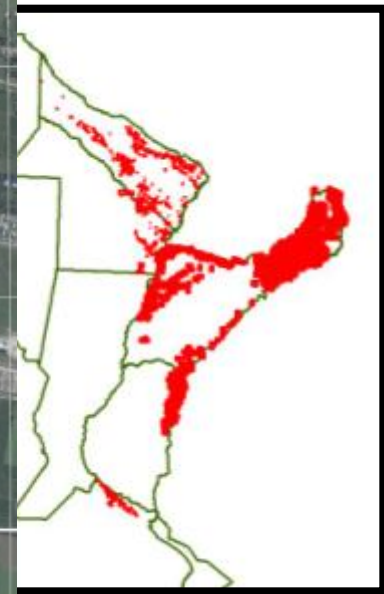
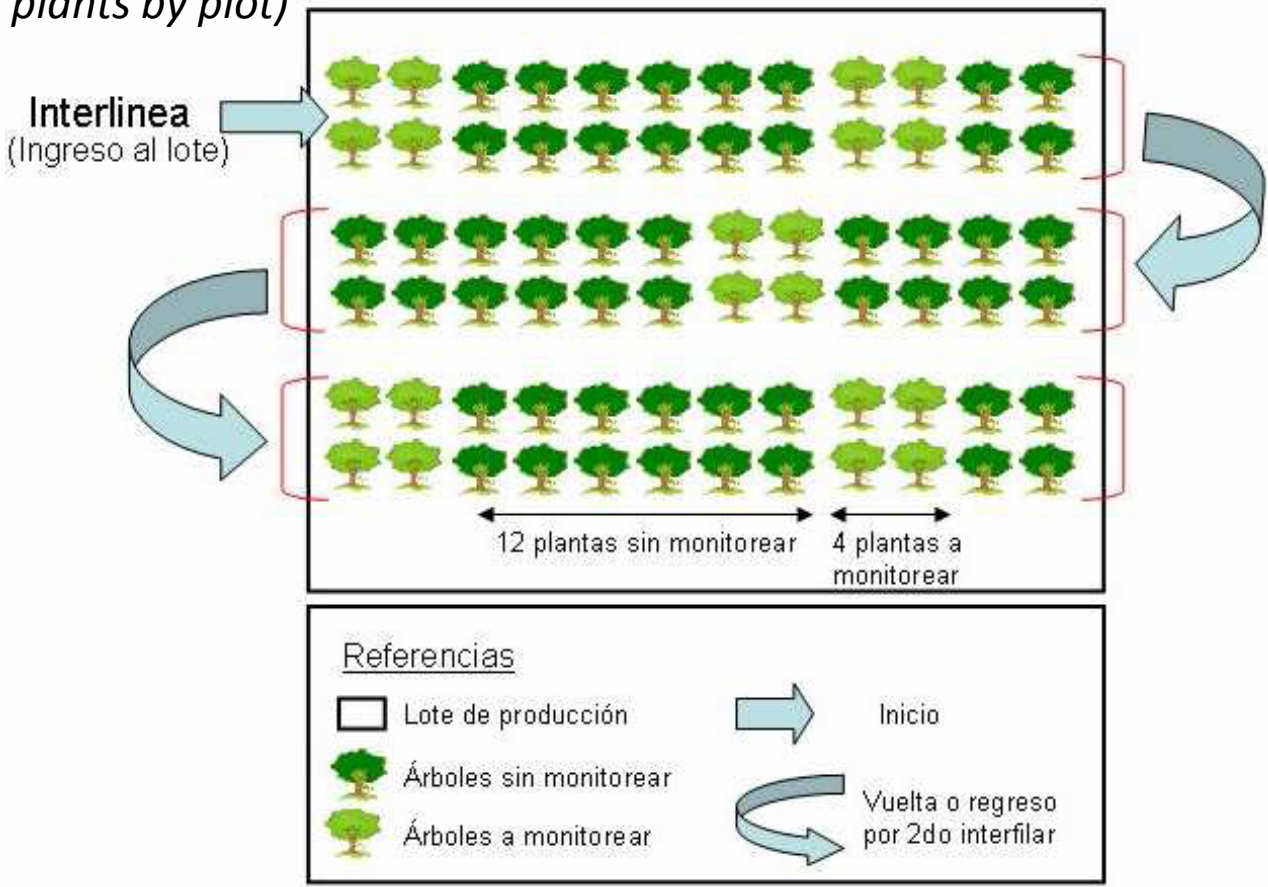


Figura I: Grilla regular y selecci

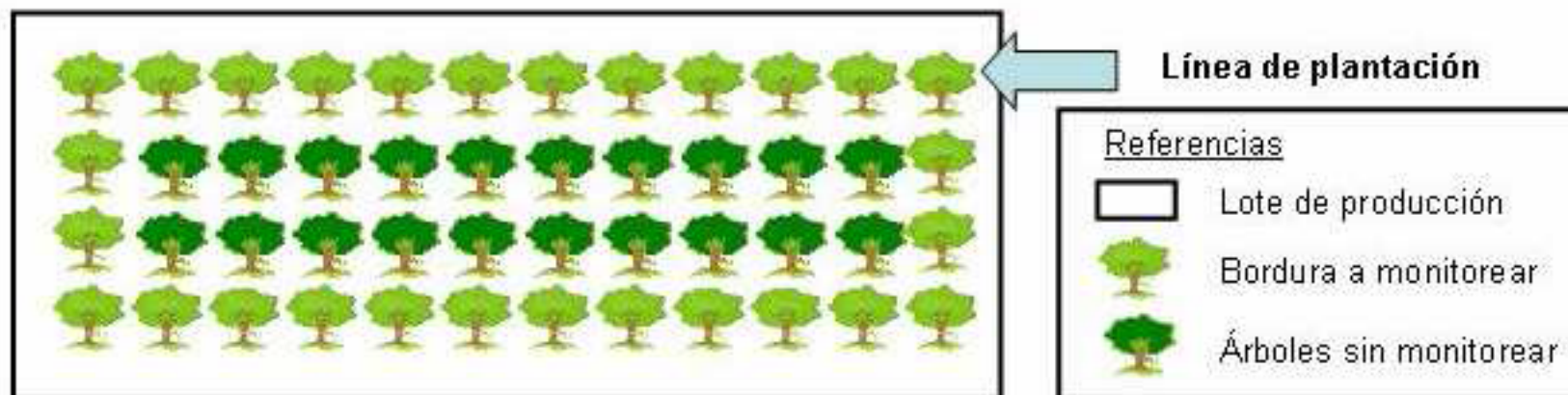
n respecto a HLB en todo el país.

Areas with presence of *Diaphorina citri*
(25% plants by plot)





Areas without presence of *Diaphorina citri*





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Sample code:
01-5655-A -1

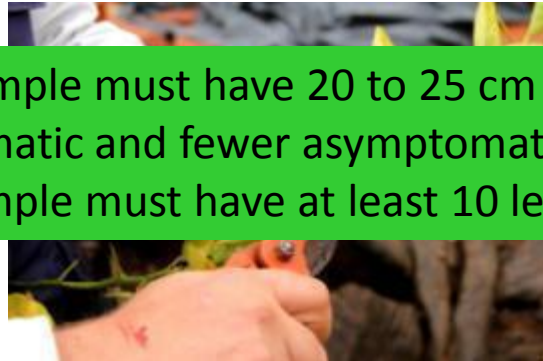




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Twig sample must have 20 to 25 cm with symptomatic and fewer asymptomatic leaves, Each sample must have at least 10 leaves



To informatics system



Vacuum pump/ catching (Areas where it is present) 5 - 10 insects adults for 1 sample to lab (alcohol 70%)



Yellow traps



1.5 – 2 m

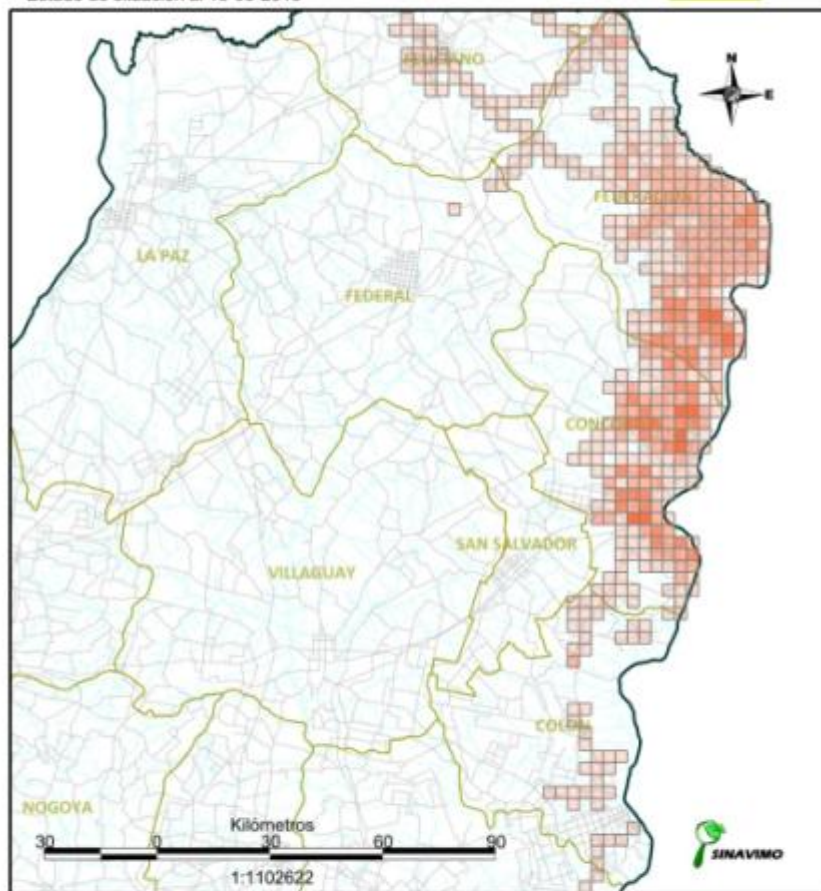


COSAVE

PROGRAMA NACIONAL DE PREVENCIÓN DE HLB

"Sitios e intensidad de monitoreo de HLB"

Estado de situación al 15-03-2013

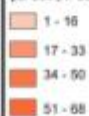


DIRECCION NACIONAL DE PROTECCION VEGETAL

PROVINCIA DE ENTRE RIOS

REFERENCIAS

Intensidad de monitoreo por sitio (N° de pasadas)



Ubicación geográfica



PROGRAMA NACIONAL DE PREVENCIÓN DE HLB

"Sitios e intensidad de monitoreo de HLB"

Estado de situación al 15-03-2013



DIRECCION NACIONAL DE PROTECCION VEGETAL
CENTRO REGIONAL
CORRIENTES MISIONES

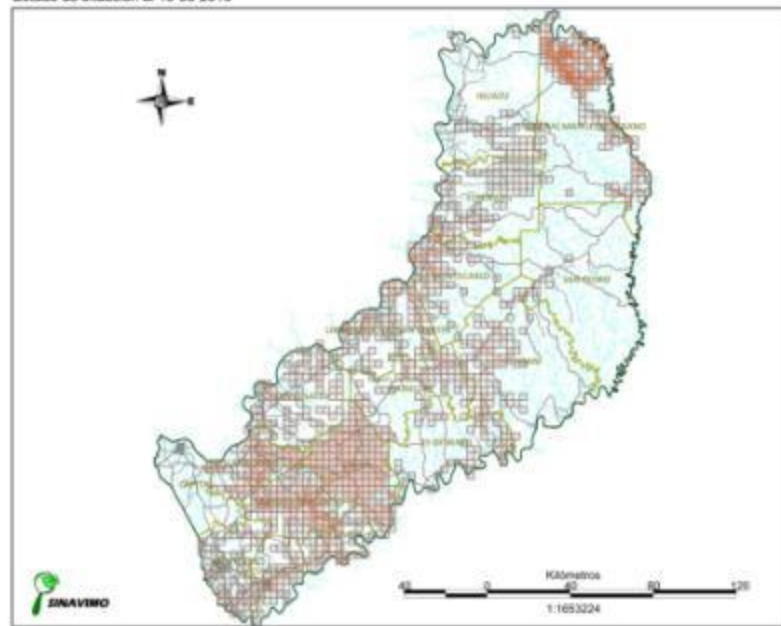
Provincia de Misiones

REFERENCIAS

Intensidad de monitoreo por sitio (N° de pasadas)



Ubicación geográfica





COSAVE

Brazil: survey

- Survey can be done on foot or using portable elevators
- N.º of surveys: 4 to 12 / year (growers)





Processing Samples in the HLB Lab



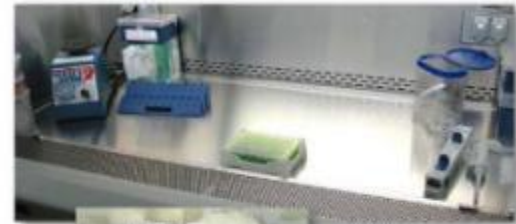
Samples arrive at lab, are entered into database, ID-numbered, stored in freezer



Sample petioles are chopped, 0.10g weighed and placed in 96-well plate with metal beads

Samples freeze-dried overnight, BeadBeat until powdered

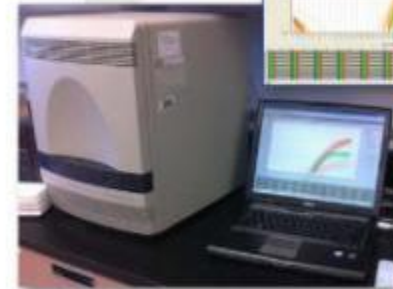
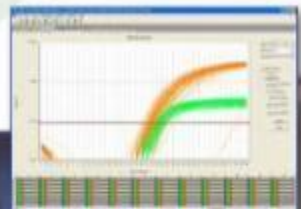
DNA extracted from samples in 96-well plate format, frozen for storage



96-well plate is run on real-time PCR machine for detecting the presence of HLB-specific gene

Sample ID	Gene	Result
101	HLB	Positive
102	HLB	Negative
103	HLB	Positive
104	HLB	Negative
105	HLB	Positive
106	HLB	Negative
107	HLB	Positive
108	HLB	Negative
109	HLB	Positive
110	HLB	Negative

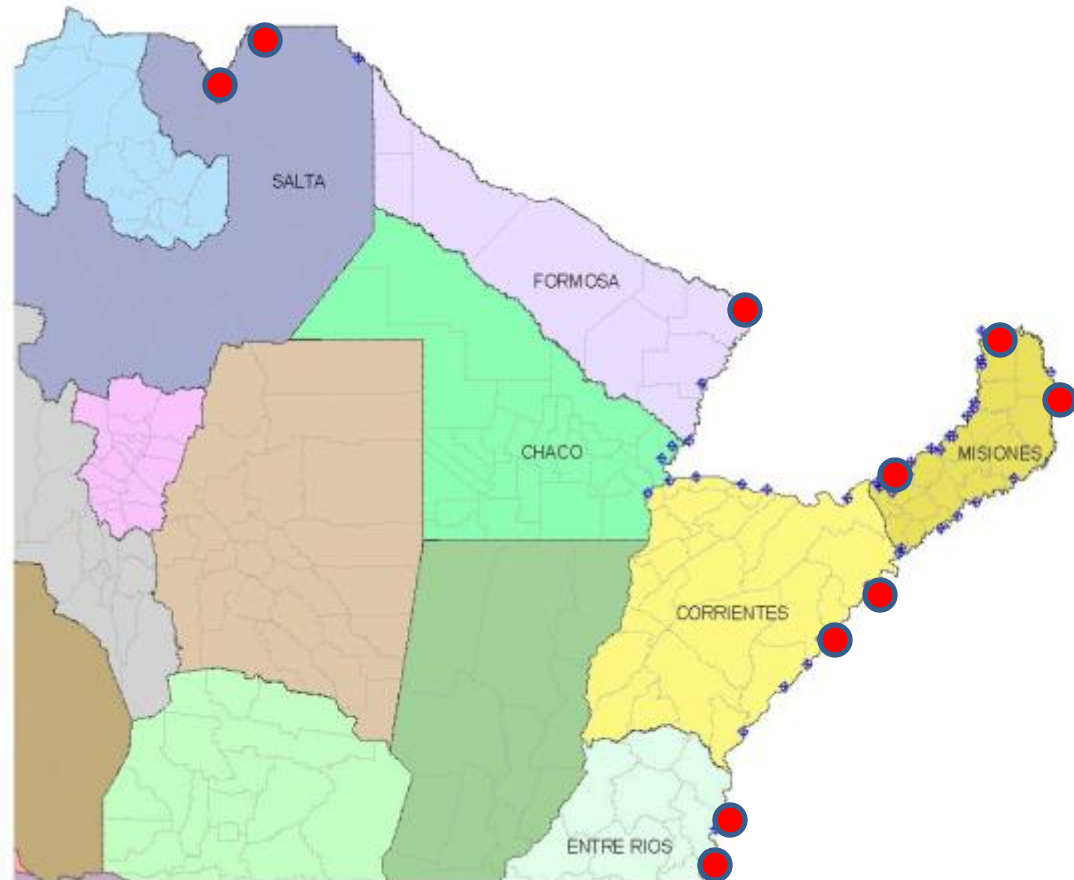
PCR results are entered into database, report generated and sent to sample submitter



Key issues to prevent



Implementing tighter control at border entrance points





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Key issues to prevent:

grow awareness in people



PROGRAMA NACIONAL DE PREVENCIÓN DEL HLB
Enfermedad que mata a los cítricos

El futuro de nuestra citricultura requiere el apoyo y la colaboración de todos para preservar el motivo: la producción de alta calidad del frutangería. Juntos podemos asegurar la sustentabilidad de la actividad y sus fuentes de trabajo.

0800-999-2386
alerta@prevencionhlb.gov.ar

CECNEA APISCA INTA senasa

Ministerio de Agricultura, Ganadería y Pesca
Presidencia de la Nación

Training : growers, technicians,
personnel of security forces





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Key issues to prevent: propagation material

- **Totally under cover production of plant propagation material**
- **Legal commerce: (only certified sanitary material must be permitted)**
- **Movement control: (identification and traceability)**





Plant propagation with officially sanitary certification



Survey and eradication of affected trees



Survey and regional vector control



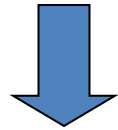


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Guidelines for Contingency Plan



Detection of a positive plant to *Candidatus Liberibacter spp.*



Eradication

Method: cut the tree trunk closest to the ground, then apply systemic herbicide to avoid reappearance

Herbicides:

- Picloran + 2,4-D
- Picloran + triclopyr
- Aminopyralid
- Aminopyralid + fluroxypyr metilheptil ester

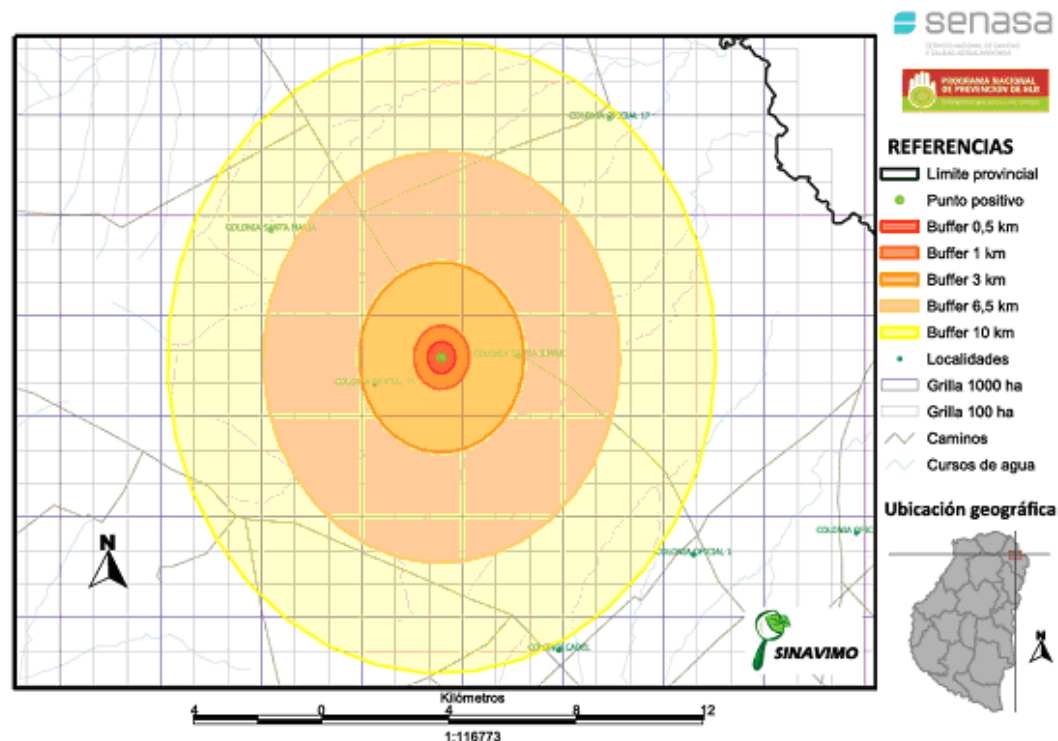


Intensification of surveys



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- Buffer zones: 0,5km; 1km; 3km; 6,5km y 10 km from the outbreak.
- Survey 100% of host in the 1st and 2nd zones
- The next areas should be surveyed at different percentages in relation with the risk and host abundance.





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Activities in contingency plan area

- Chemical control of *Diaphorina citri*



- Awareness of people in the area





MUCHAS GRACIAS

