

This slide show on the greening disease of citrus that you are about to see has serious significance for the future of the citrus industry of Mexico.

We will talk about a disease which has destroyed and is currently destroying citrus industries worldwide.

Este presentación que van a ver sobre la enfermedad “greening” de los cítricos tiene mucha significancia por el futuro de la industria citrícola de Mexico.

Platicamos sobre una enfermedad que ha destruido y ya está destruyendo industrias citrícolas por todo el mundo.

THE GREENING DISEASE OF CITRUS

Unquestionably the most serious and destructive disease of citrus

- There is an old saying among plant virologists: “When greening comes in, citrus goes out.”
- More than ever, this seems to be true today!



Citrus Greening Disease (Huanglongbing -HLB)

- Destroyed several citrus industries in Asia and Africa
- Has **NOT** been effectively managed anywhere so far



Green = HLB and psyllids both occur
Orange = psyllids occur
(Also in Texas)

Huanglongbing means yellow shoot disease
We will use “greening disease” in this slide show

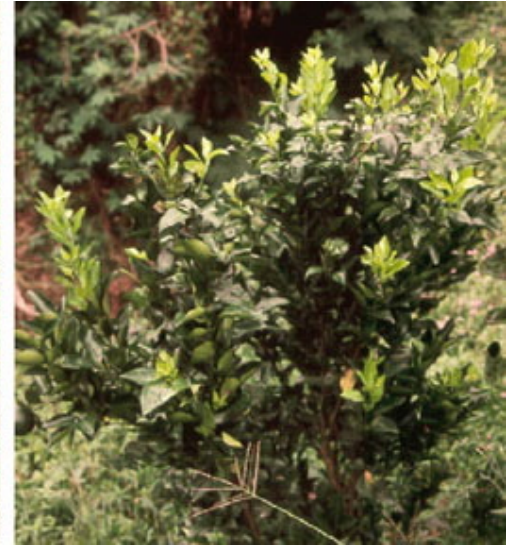
The greening disease of citrus

We will review:

- **Symptoms world wide**
- **The organisms**
- **The vectors**
- **Detection**
- **Epidemiology and Control**
- **Conclusions**

SYMPTOMS WORLD WIDE

- Symptoms of greening found south of Taipei, Taiwan in 1981. Notice yellow shoots. In Taiwan the disease was called 'likubin' (drooping disease).



- Removing infected trees in Taiwan in 1969. Currently the fate of many groves in greening troubled areas throughout China, Indonesia, Malaysia, Thailand and Southeast Asia, and now in Florida and Brazil.



SYMPTOMS WORLD WIDE

Greening (HLB) in Thailand

Typical greening infected tree

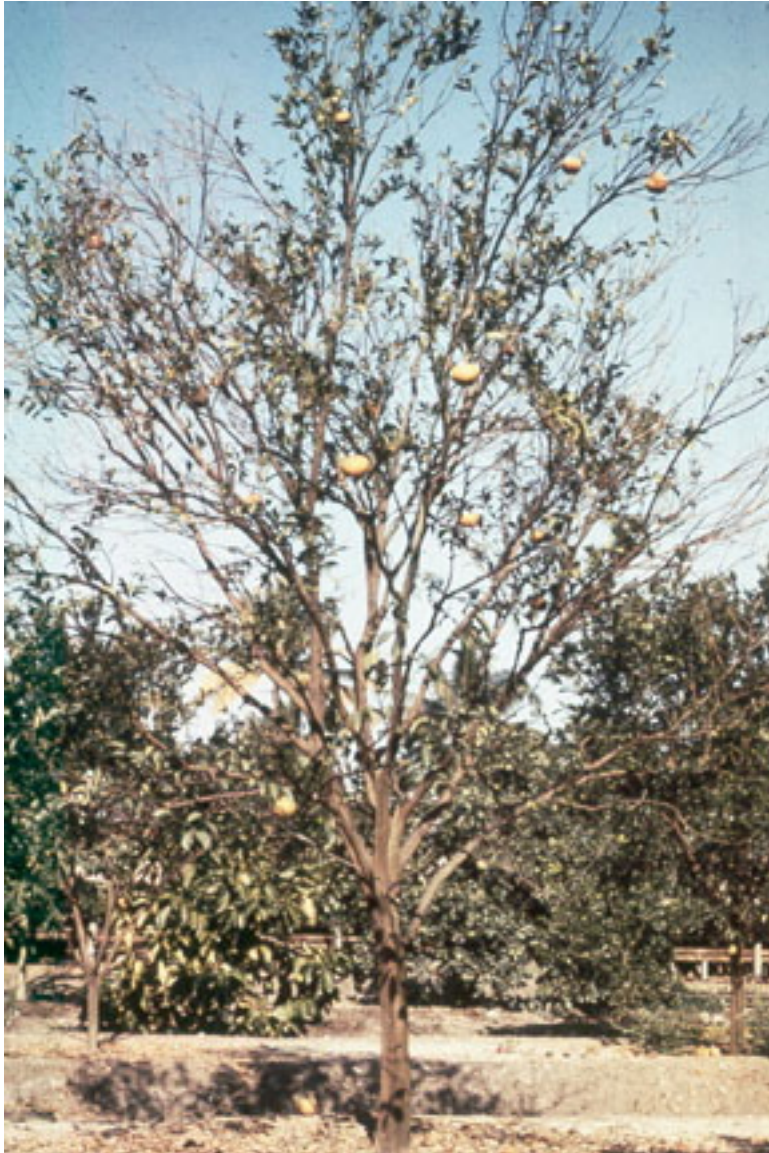


The only healthy tree found in Thailand



SYMPTOMS WORLD WIDE

Dying tree in Thailand



In 1995 students and faculty in Thailand would not believe that this picture of a dying tree was taken in Thailand. They said the tree was too big and that there are no such big trees in their country!

In truth, the picture was taken in 1975 by Dr. Calavan and most people in the audience had never seen a large citrus tree in Thailand.

SYMPTOMS WORLD WIDE

Greening (HLB) in New territories, Hong Kong

- **A mandarin showing classical symptoms of greening in 1969. Many trees in the region of the New Territories showed these typical symptoms of yellow shoot Chinese name (Huanglongbing)**



SYMPTOMS WORLD WIDE

Greening (HLB) in India

- **In 1992, many groves visited in India were infected with greening**
- **A Cong mandarin in a grove near Gonicopol, India.**



SYMPTOMS WORLD WIDE

Greening (HLB) in South Africa

- **A typical greening infected tree at the Letaba Estates near Nelspruit, South Africa in 1977. Note the fruit drop and green color of fruit on the ground. Because of this classic fruit symptom, the disease was named `greening`.**



SYMPTOMS WORLD WIDE

Stylar end greening

- Stylar end greening on samples of various fruit infected with HLB. The infected fruit is contrasted against non-infected normal fruit. (Note the greening at the stylar end in contrast to the normal orange color).

(Slide of Monique Garnier)



SYMPTOMS WORLD WIDE

Leaf mottle typical for greening disease

- **Greening-infected tree of Valencia orange at the Letaba estates in South Africa 1977 showing yellow midribs, leaf mottle and general chlorosis of leaves.**



SYMPTOMS WORLD WIDE

Leaf mottle typical for greening disease

- Various types of leaf mottle associated with HLB. Note the asymmetrical type of leaf pattern (lower left).**

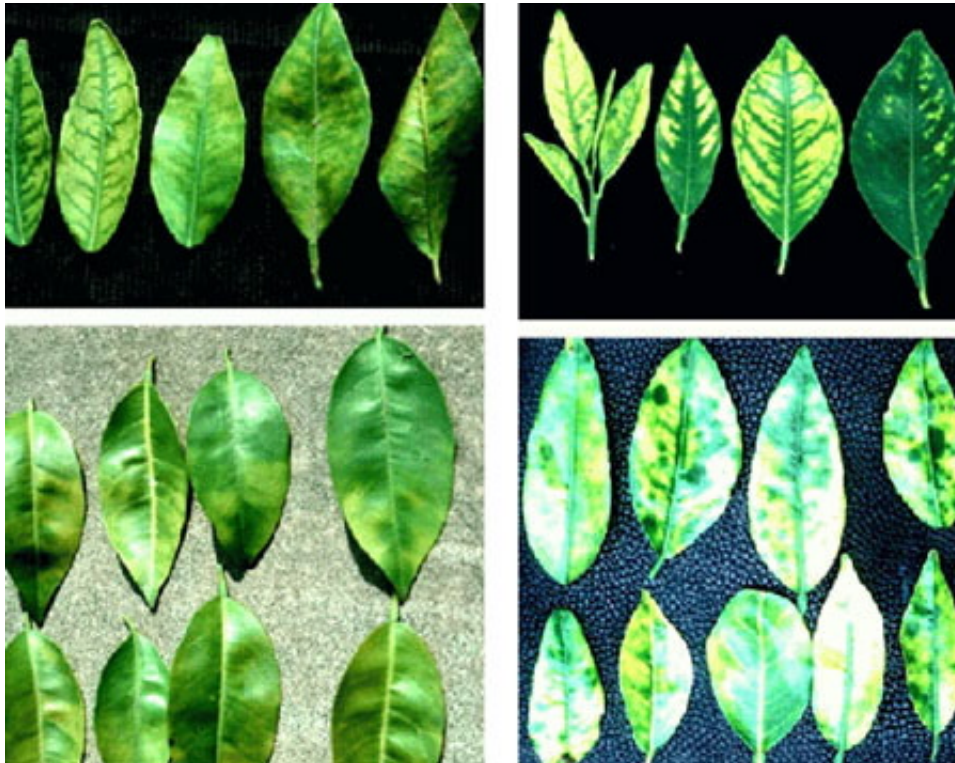


Photo Monique Garnier

SYMPTOMS WORLD WIDE

Vein corking in India

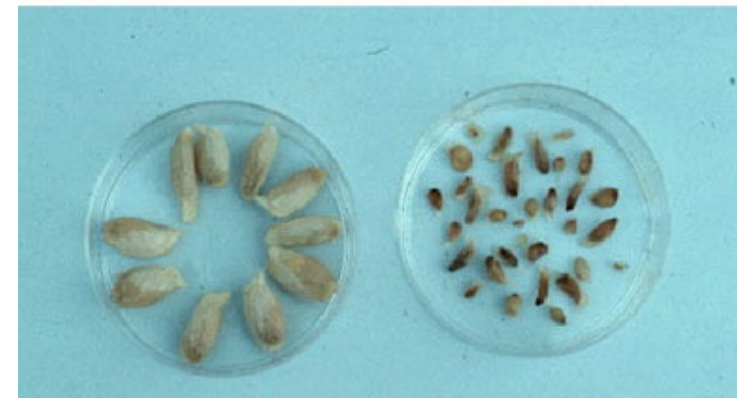
- **Vein corking on sweet orange associated with HLB. This symptom can readily be confused with CTV vein corking symptoms. Photos taken in the Hindispur region of India.**



SYMPTOMS WORLD WIDE

Seed abortion

- **Classical seed abortion from fruit of an HLB infected sweet orange tree. Note the small purple colored seeds contrasted with normal seeds. This is also typical for stubborn disease.**



SYMPTOMS WORLD WIDE

Greening (HLB) in Florida

- **First reported and confirmed in Florida in September, 2005. The vector *Diaphorina citri* had been present in Florida since 1998.**



Greening (HLB) in Brazil will kill trees in 3 to 5 years



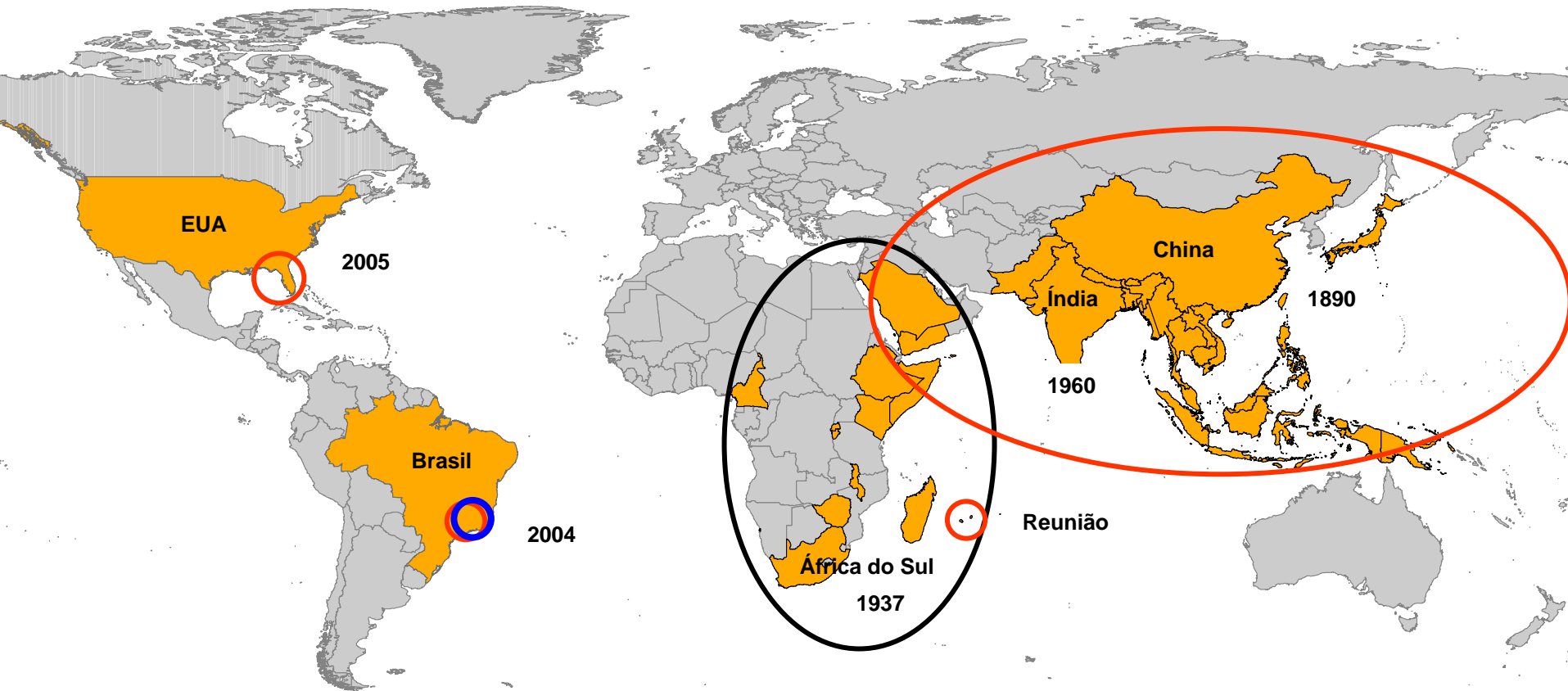
3 to 5 years

The organisms

- The greening organisms are fastidious phloem limited bacteria in the genus *Candidatus Liberibacter*.
- The bacterium has not been cultured to date and Asian, African or Brazilian bacteria all look alike as shown below.



World geographic distribution of three types of bacteria



Slide - Silvia Lopes

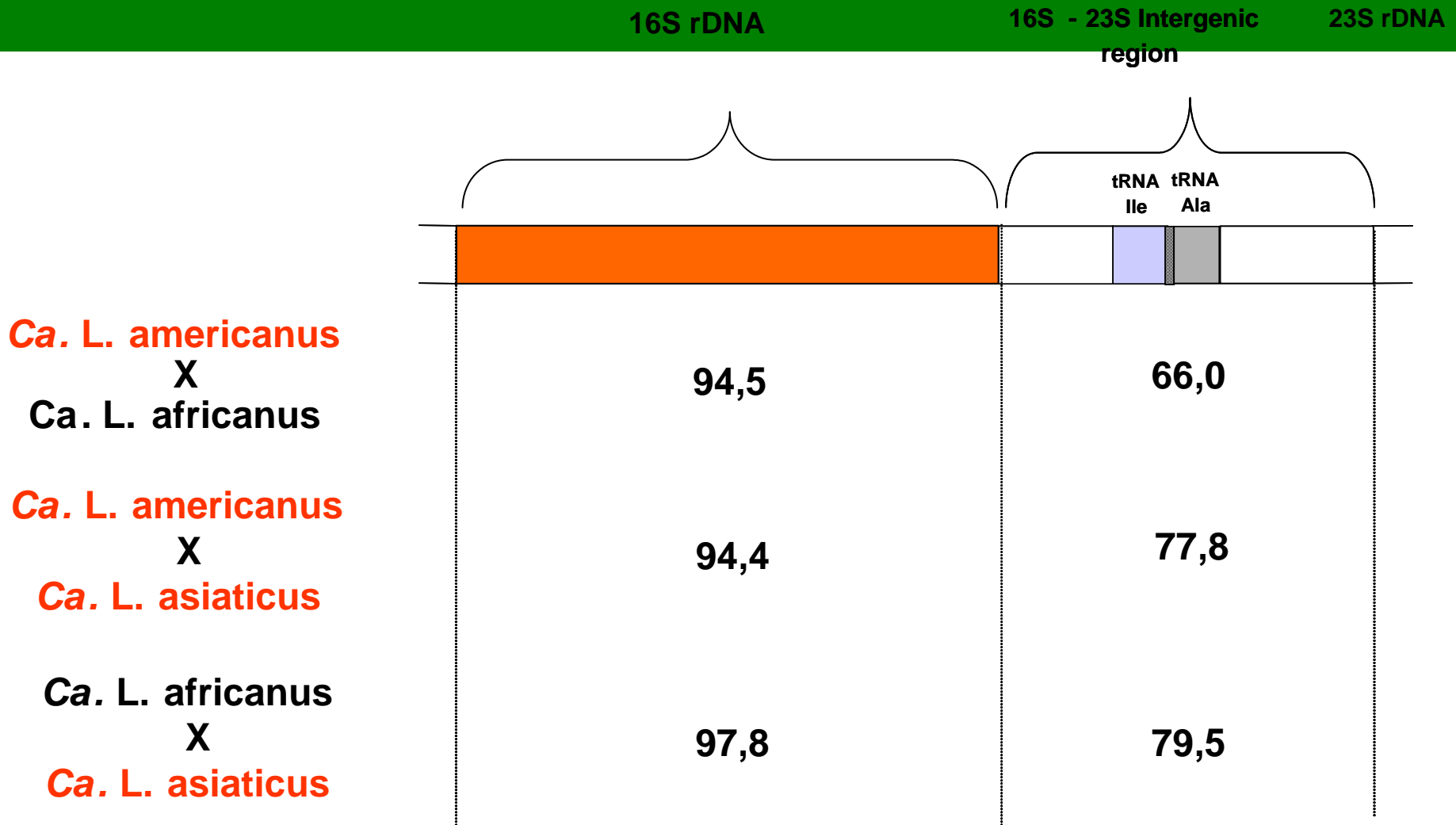
***Ca. Liberibacter asiaticus* (25 countries)**

***Ca. Liberibacter africanus* (12 countries)**

***Ca. Liberibacter americanus* (Brazil)**

Identities among the *Ca. Liberibacter* species

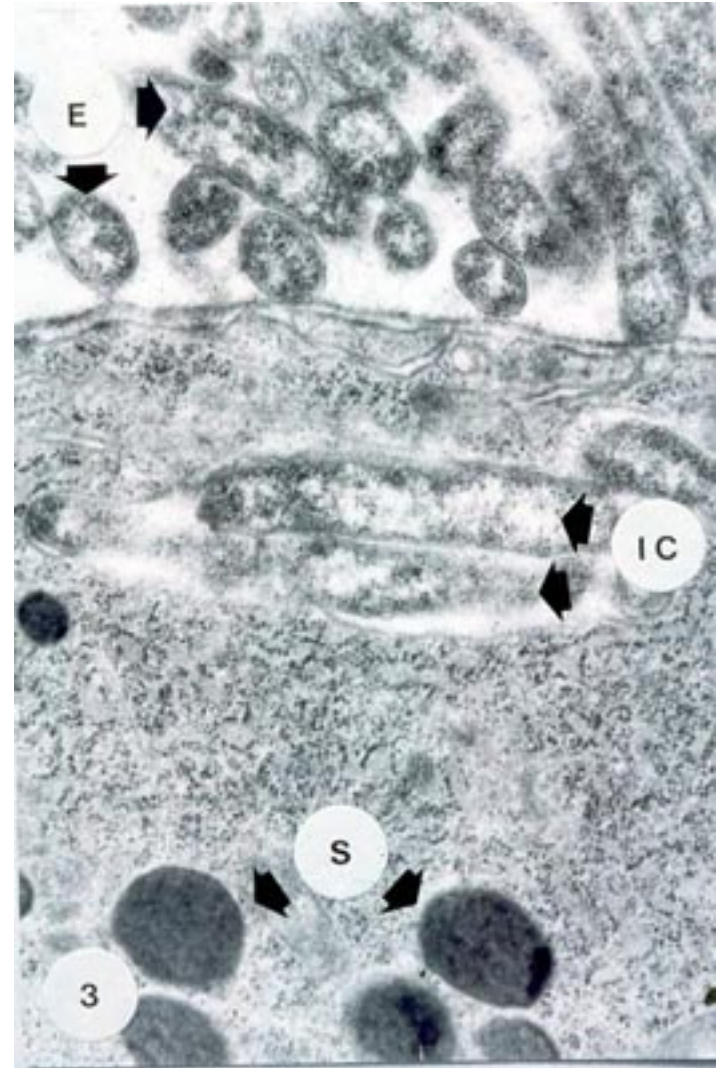
Three distinct species! (Slide – Silvio Lopes)



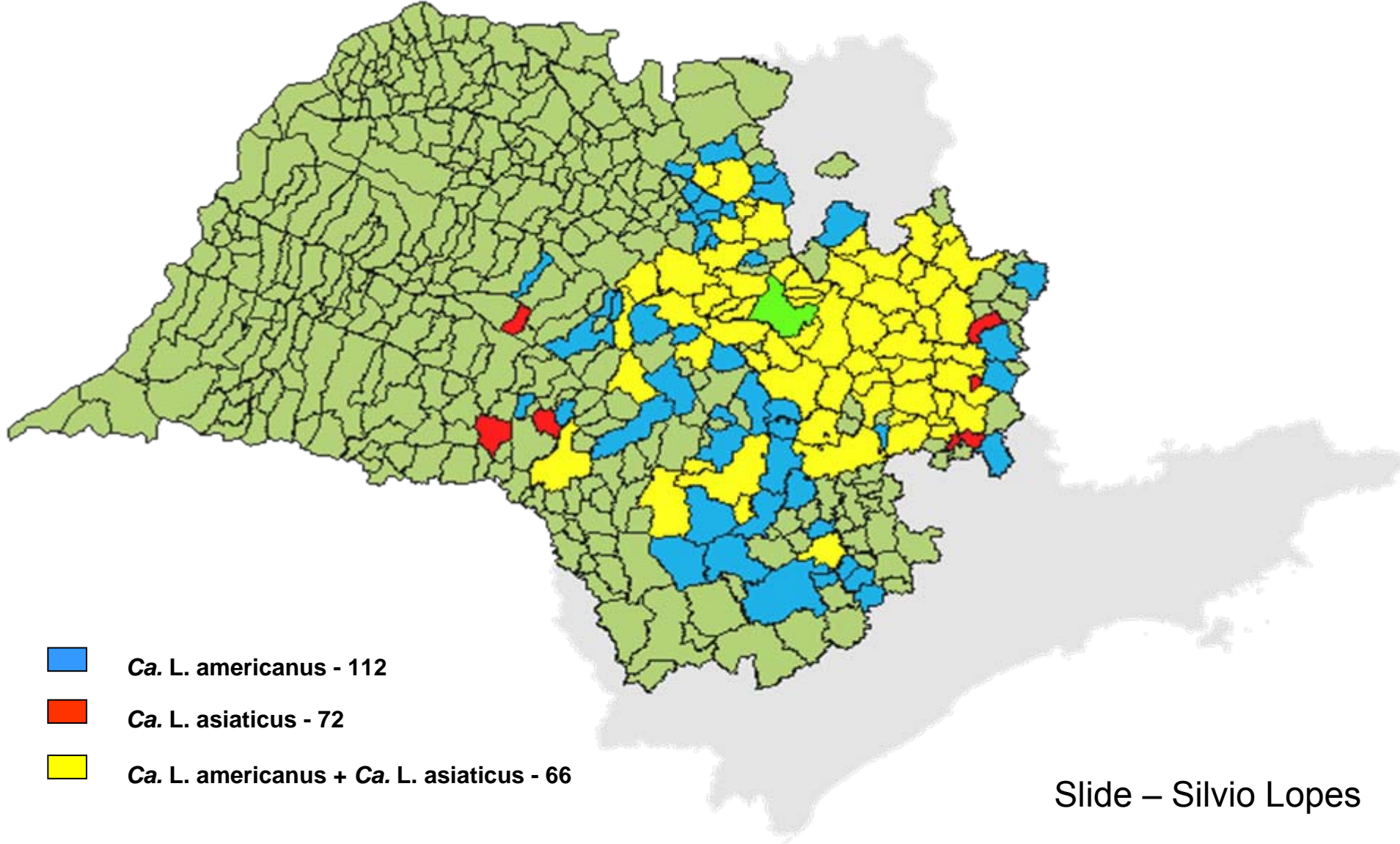
The greening bacteria inside the vector

- **Abundant greening bacteria found in the haemolymph of *Trioza erytreae*. Acquisition by the adult vector within 5 days of feeding. The bacteria enters the salivary glands in 21-20 days and proliferates in the haemolymph and the vector becomes infective.**

From Moll and Van Vuuren (1977)



Current distribution of 2 types of greening in São Paulo State



Slide – Silvio Lopes

Detection
of the organism
and
the disease

Citrus Greening Disease

Asymmetrical, lopsided fruit with aborted seed

Candidatus Liberibacter asiaticus
Ca. L. americanus and *Ca. L. africanus*

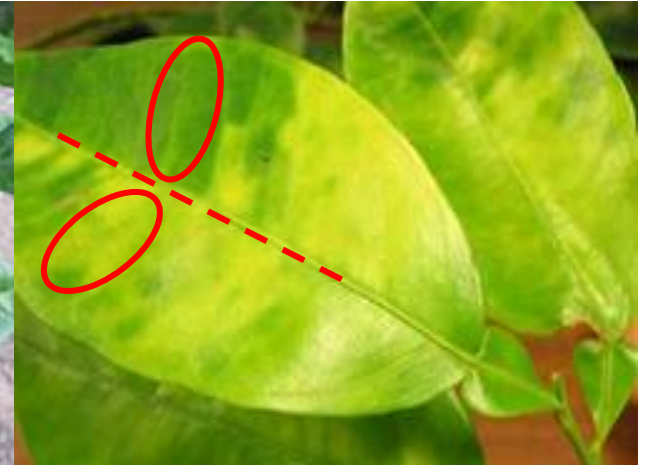
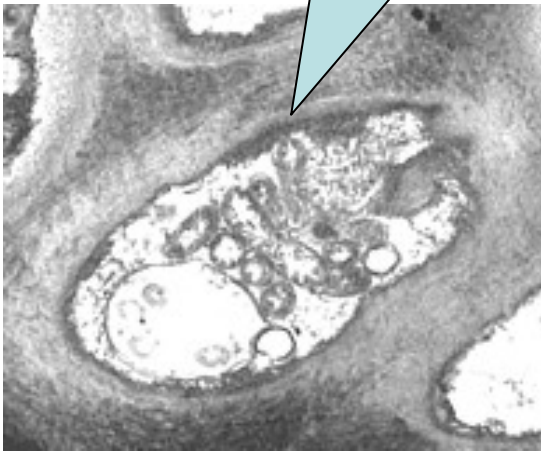
- The bacteria can be confirmed to be present by PCR from
- Difficult to culture Bacterium limited to the phloem, not cultured so far, thus the conditional name *Candidatus*
- Long latent period of Liberibacter
- Long latent period at low temperature.

Bacterium limited to the phloem, not cultured so far, thus the conditional name *Candidatus* Liberibacter

trees /
of



Asymmetrical blotchy mottle leaf symptom common and used for diagnostic PCR assays



Field detection of greening disease



Stunted tree

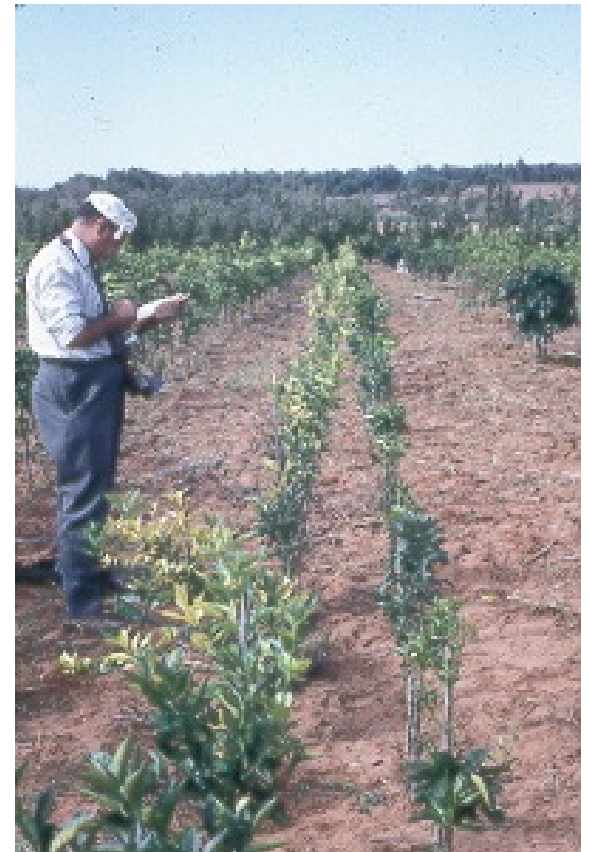
Yellow shoot

Leaf mottle

Fruit greening



Is this greening disease? Observed in Morocco in 1966



Are these due to greening disease?

Left – in Nepal

Right – in the Fiji Islands

We must index to be sure!



We thank Eduardo Carlos for
some of these slides
presented at the 17th IOCV
Conference in Adana, Turkey
– October, 2007



Current status of citrus Huanglongbing (HLB) in São Paulo State, Brazil, based on molecular and visual diagnosis.

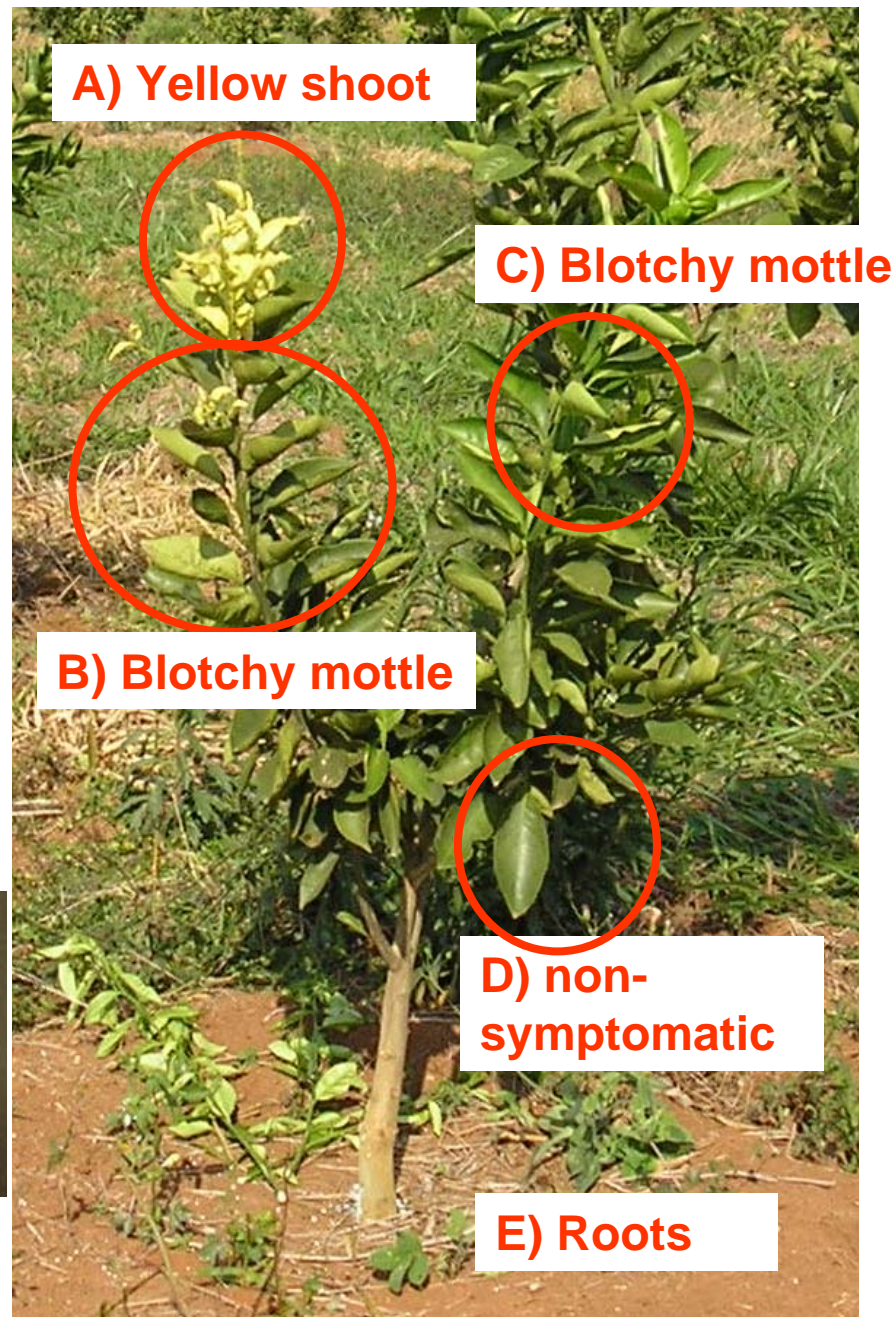
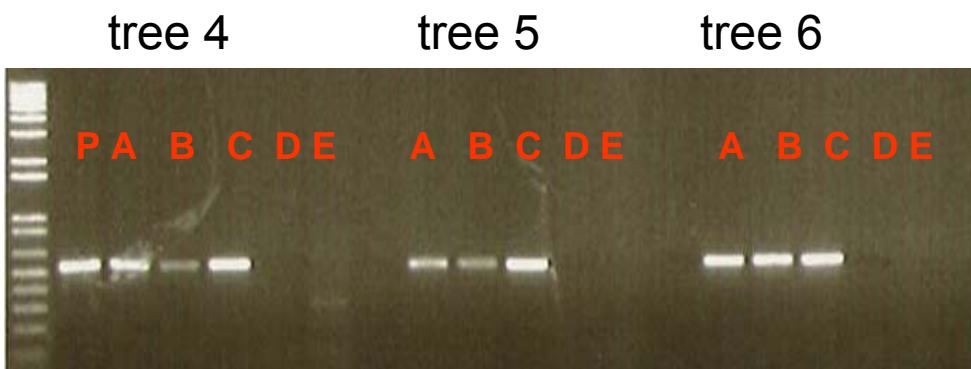
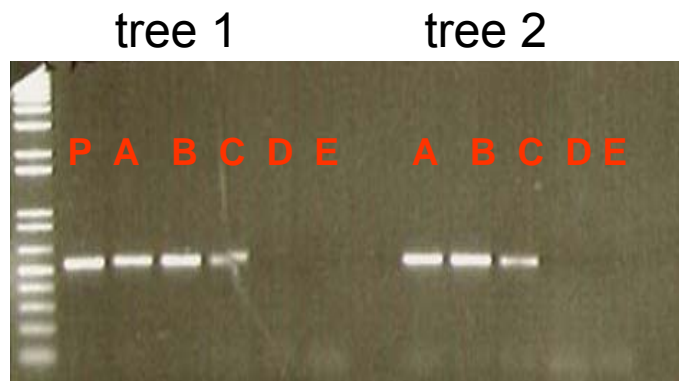
Eduardo F. Carlos; Helvécio D. Coletta-
Filho; Luiz L. Lotto; Luciane F. Coerini;
Marcela T. Vitorino; & Marcos A. Machado /

Cordeirópolis, SP, Brazil

efcarlos@centrodecitricultura.br

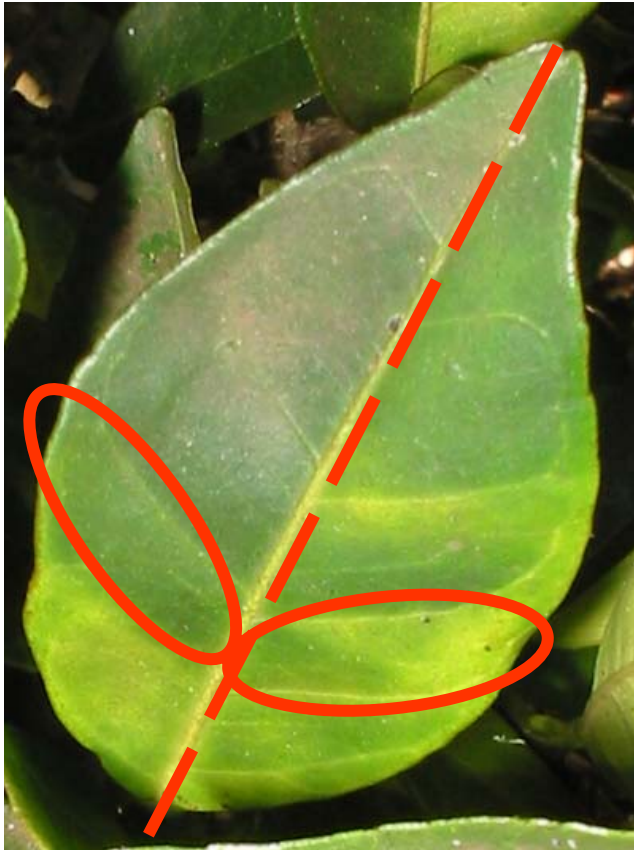


Detection methods



Field diagnosis for surveys on a large scale

Blotchy mottled leaf



Asymmetrical chlorosis

“the pen method”



- Can we use PCR for massive detection of 80 million trees in Brazil?
- Cost of PCR
- Practical to confirm field diagnosis
 - Estimated cost US\$ 20.00 / PCR reaction
 - Total cost of near US\$ 1,600,000,000.00 (above 1 billion !!!)



Training scouts for Fundecitrus and CDA (2005)



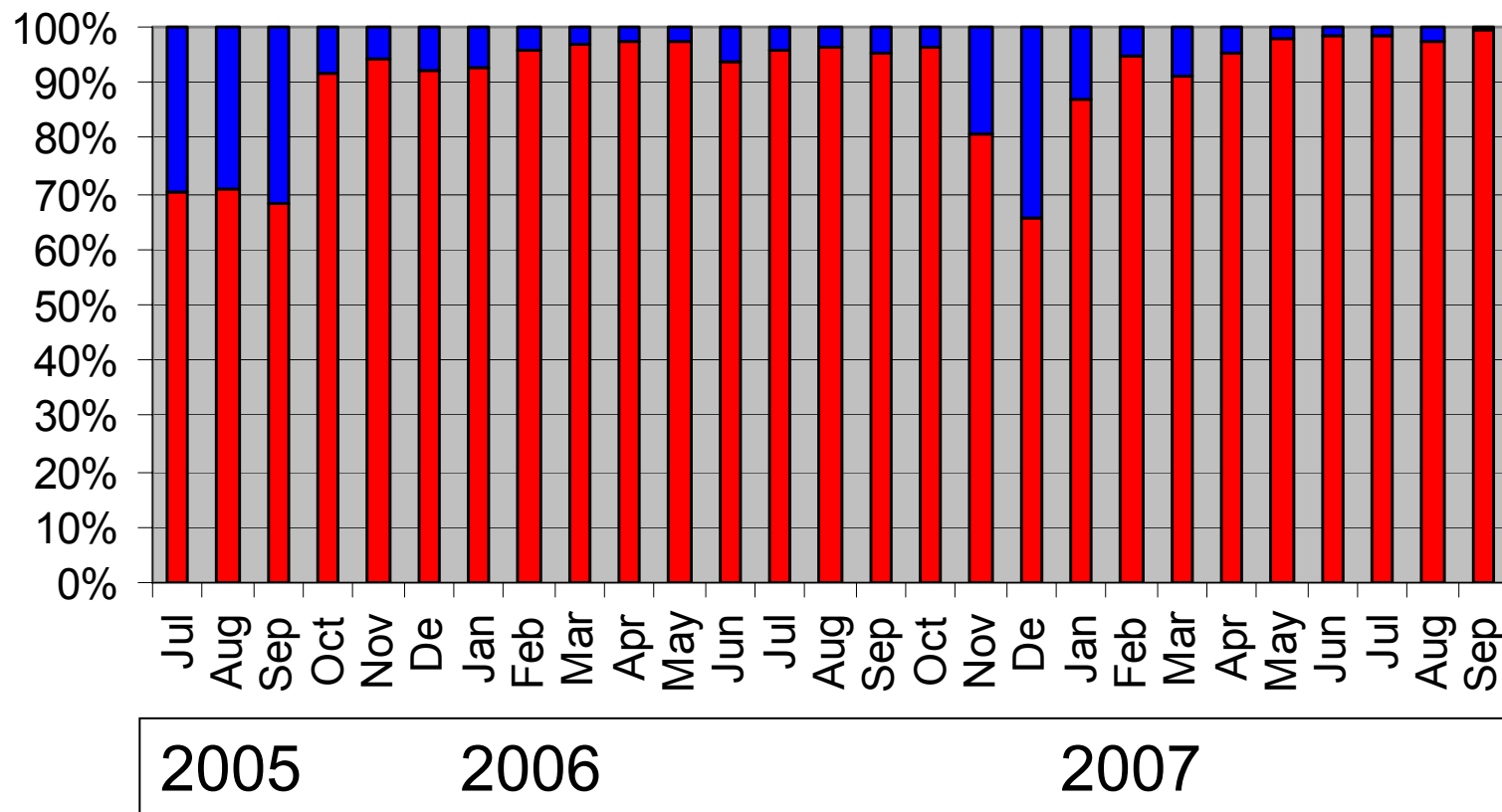
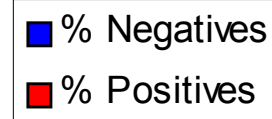


Centro
APTA Citros
Sylvio Moreira IAC

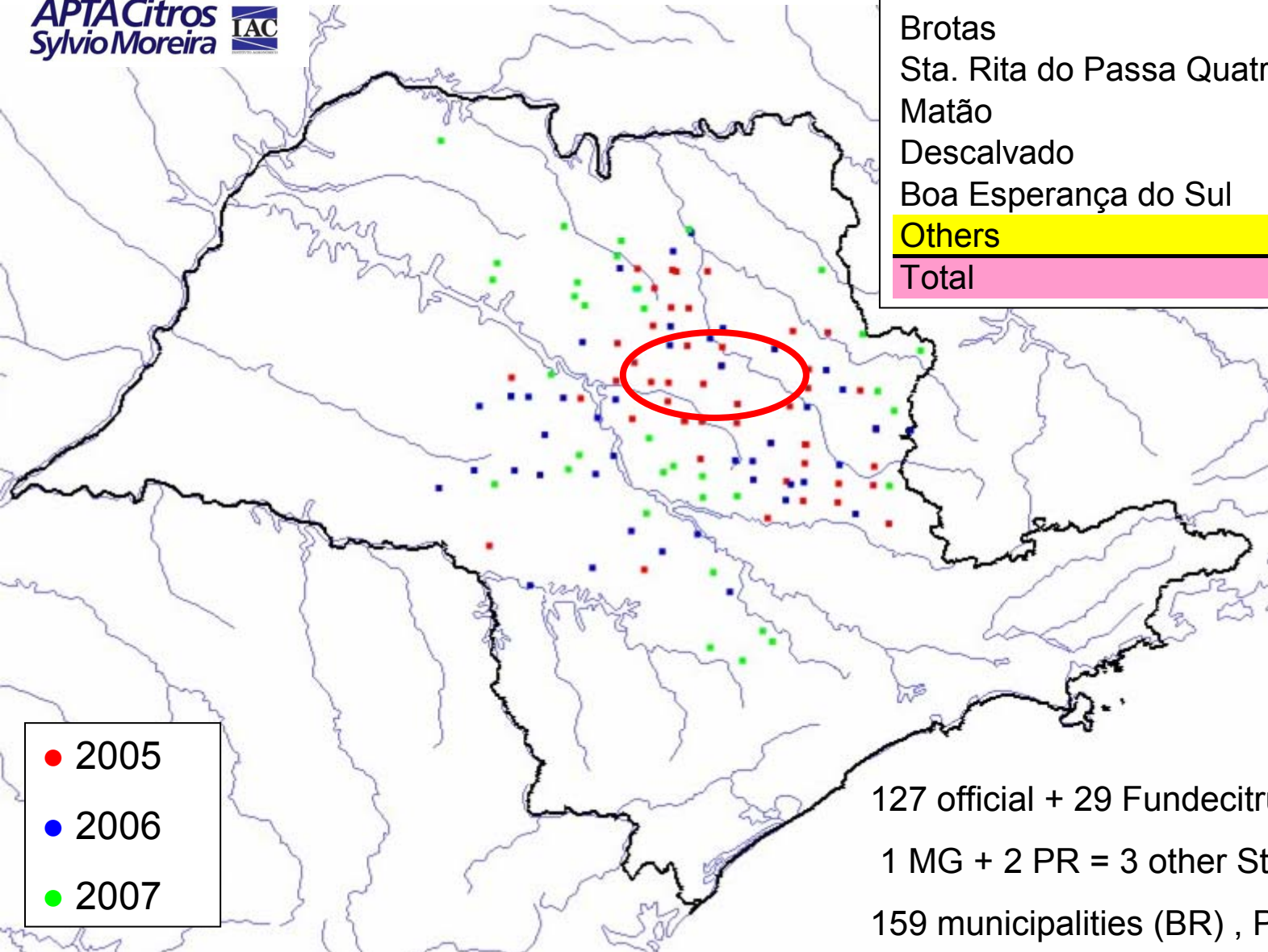
Efficiency of HLB diagnosis

Samples (trees) processed at the Sylvio Moreira Citrus Center

Samples for HLB



Municipalities	% Total
Araraquara	32.7
São Carlos	10.0
Brotas	6.8
Sta. Rita do Passa Quatro	6.3
Matão	6.2
Descalvado	5.7
Boa Esperança do Sul	3.9
Others	28.5
Total	100.0

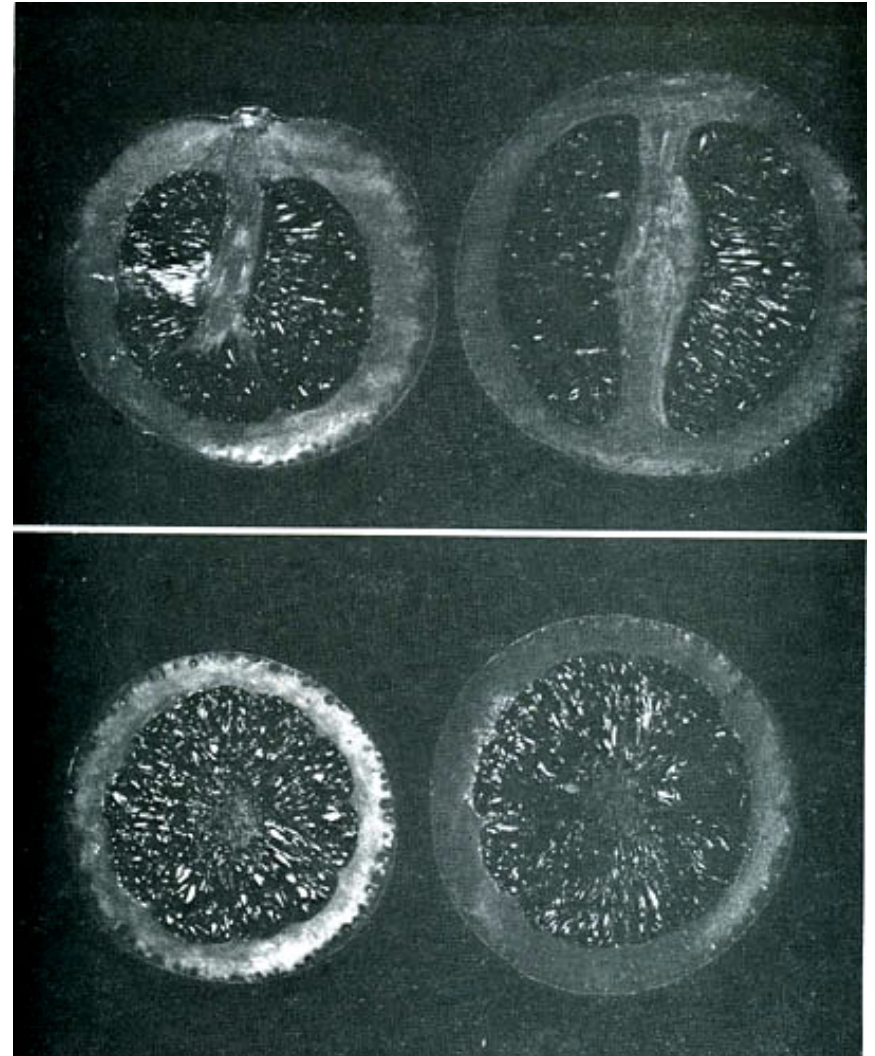


● 2005
● 2006
● 2007

127 official + 29 Fundecitrus = 156 SP
 1 MG + 2 PR = 3 other States
 159 municipalities (BR) , PCR +

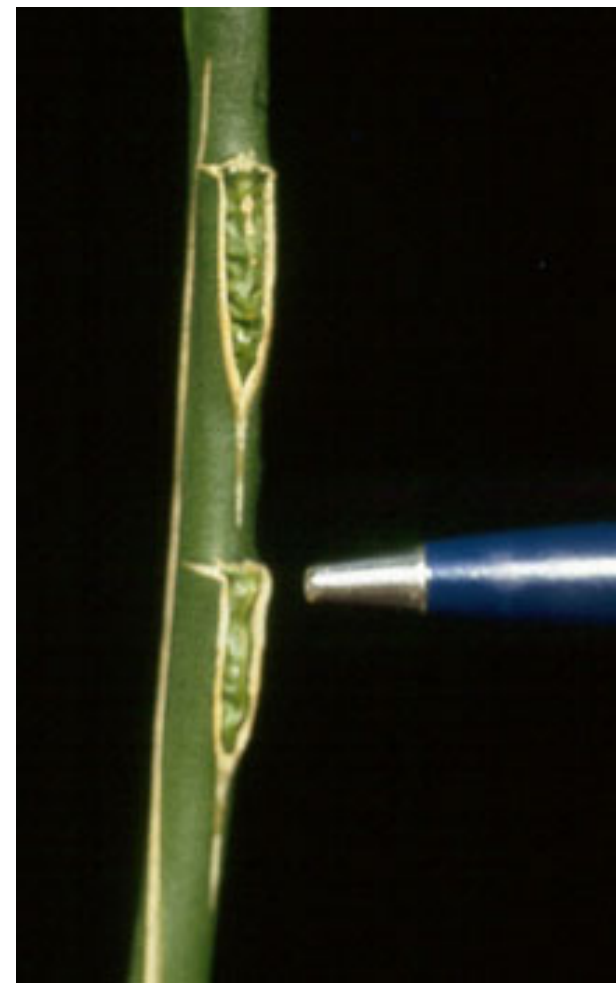
Fluorescent markers - an early index for greening disease

- Schwarz (1968, [1970](#)) developed an albedo fluorescent index for detection of greening disease and showed seasonal variation. The fluorescent marker test was used to identify greening disease in Nepal, in West Pakistan; in Thailand; in Ethiopia; in Kenya; in South Africa; in the Reunion Islands and is currently in use in Brazil.



Graft transmission: Side grafts or leaf grafts are superior to bud grafting.

Graft transmission is useful for positive diagnosis – but is long term and not practical for massive detection.



Warm temperatures suppress symptom development of South African greening.

- When inoculated seedlings of sweet orange were kept at 21C, clear leaf symptoms developed as shown in the center.
- However, if inoculated plants were moved and maintained at 26-28C, no symptoms appear on the new growth as shown on top



Detection of the greening organism can be done by PCR.

Current technology can detect the greening organism inside the insect vector before symptoms appear in field trees (Manjunath et al. 2007)



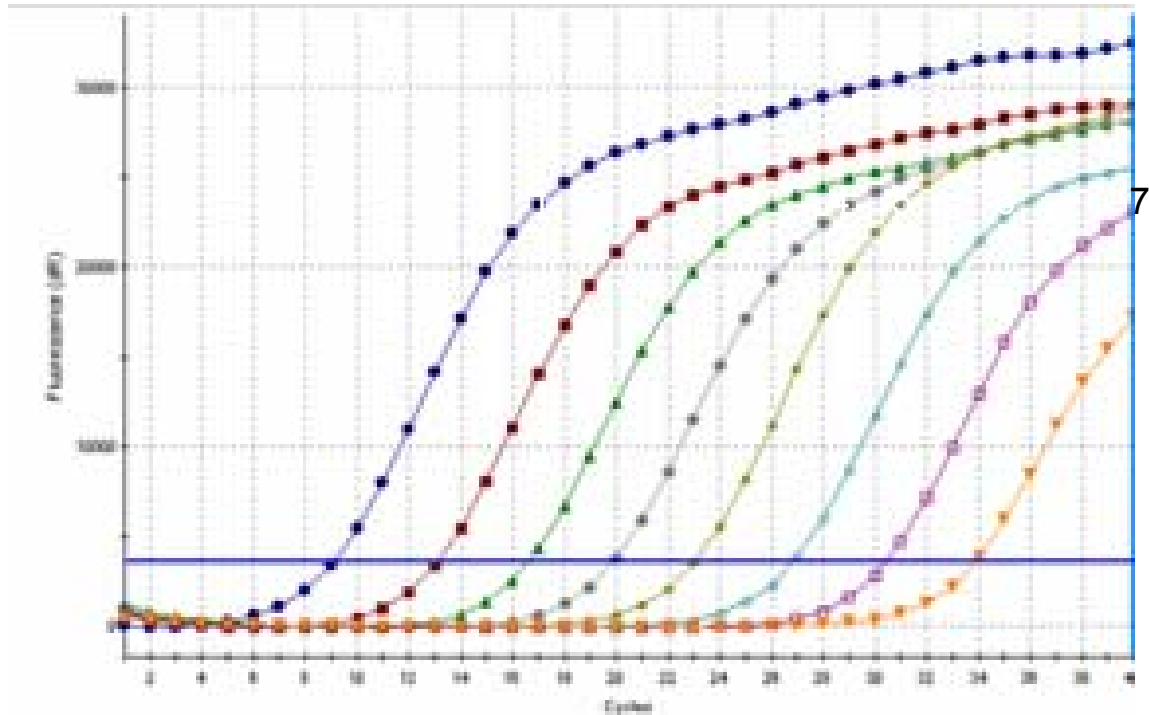
REGULAR PCR



REAL TIME PCR

Real time PCR Detection of HLB

Plasmid dilution	Ct (dR)
10^{-1}	9.08
10^{-2}	13.1
10^{-3}	16.63
10^{-4}	19.88
10^{-5}	23.04
10^{-6}	26.83
10^{-7}	30.44
10^{-8}	33.9



❖ A plasmid DNA with HLB target sequence (16s rDNA) was used as template

The advantages of detecting the bacteria in the DNA of the psyllid

Monitor HLB in Asian citrus psyllids

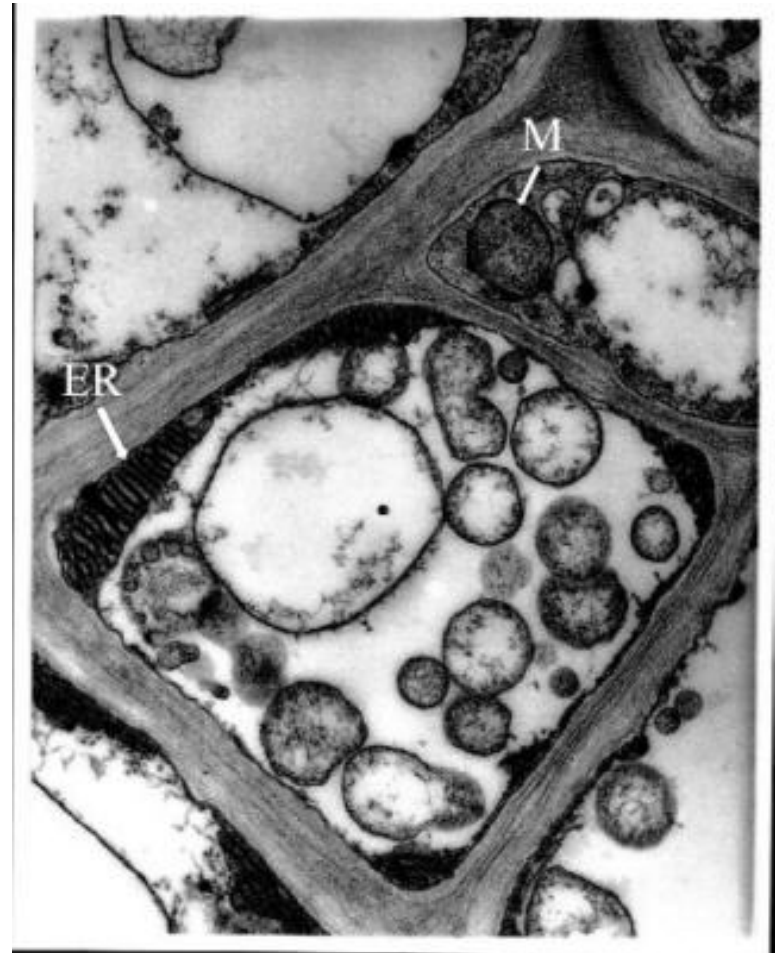
- ❖ Prolonged latency in Plants: disease is detectable in plants only after several months to years after infection
- ❖ Psyllids live for 50-70 days. The greening bacteria can reach very high titers within 1-2 weeks
- ❖ HLB positive psyllids can be found in an area one to several years before onset of symptoms on plants



There is evidence of a new organism which can induce greening symptoms in field trees but which is a phytoplasma (Bove et al. 17th conference IOCV)

- “This new HLB-associated phytoplasma is closely related to the Pigeon pea witches’ broom phytoplasma (group 16Sr IX) with 99% maximum identity.”

J.M. Bove



A new phytoplasma disease in Brazil

Leaf symptoms are identical to those of greening disease

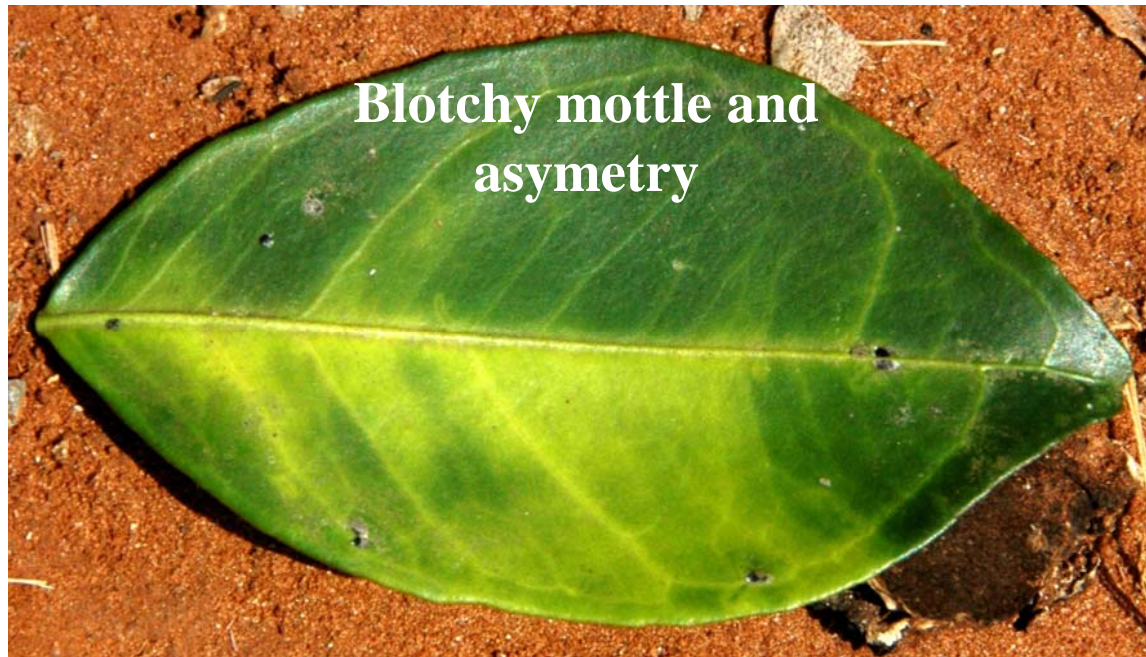
Blotchy Mottle on sweet orange leaves from Barretos, SP.



From J.M. Bové

A new phytoplasma disease in Brazil

The asymmetry in the leaf is identical to that of greening disease



From J.M. Bové

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A new phytoplasma disease in Brazil

The lopsided fruit and seed abortion symptoms are identical to those with greening disease

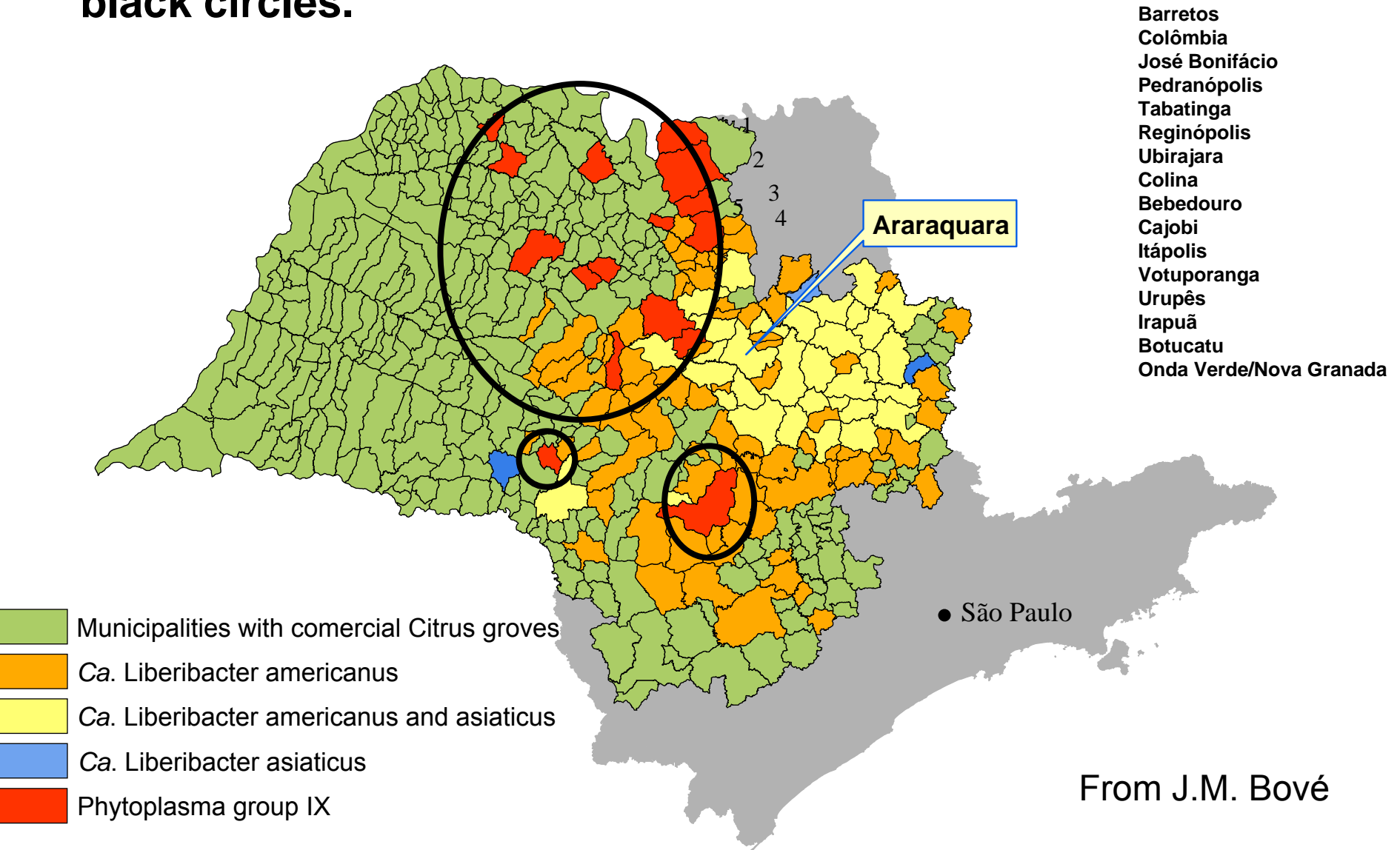
Lopsided fruits with aborted seeds and brownish vascular bundles



From J.M. Bové

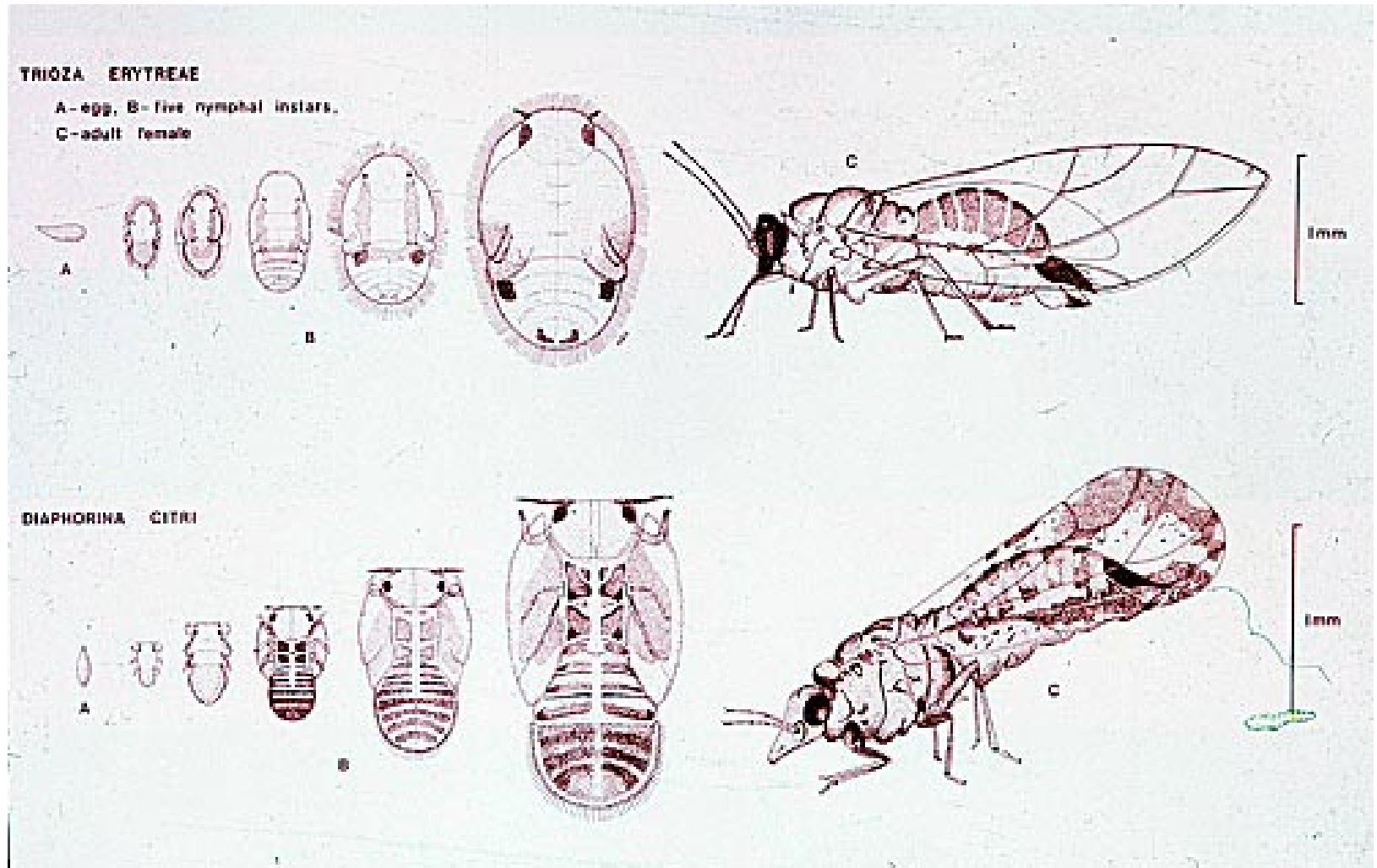
Municipalities with Huanglongbing in São Paulo State.

The 16 municipalities with HLB phytoplasmas are within black circles.



From J.M. Bové

The Vector – a psyllid



The two vectors of greening disease

Diaphorina citri (left) vector for Asian greening
(note the characteristic angle of feeding)

and

Trioza Erytreae (right) vector for African greening



Trioza erytreae

Showing eggs nymphs and leaf distortion



Showing the distribution of *Trioza erytreae*

- *Trioza erytreae* can be found throughout much of Africa south of the Sahara. It is also found with *Diaphorina citri* in Saudi Arabia, Yemen, in Mauritius and Réunion. It has also been reported in the Madeira islands.

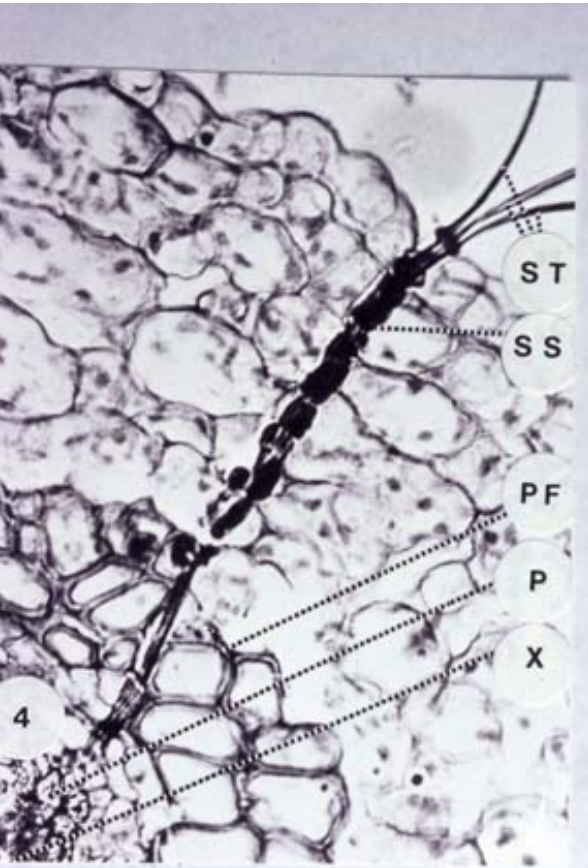


D. Citri – the adult, eggs and nymphs

- **D. Citri eggs are laid on "feather flush" and hatch in 2 to 4 days. There are five nymphal instars which are completed in 11 to 15 days. The total life cycle takes 15 to 47 days, depending upon the temperature. Adults may live several months and the females lay as many as 800 eggs in a lifetime. The optimum development temperature range was found to be 25-28C.**

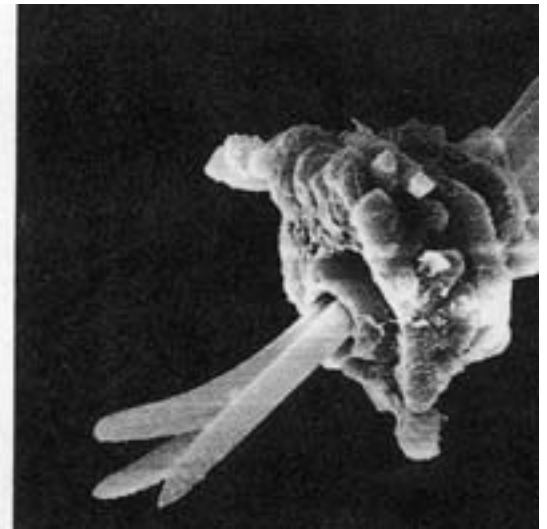


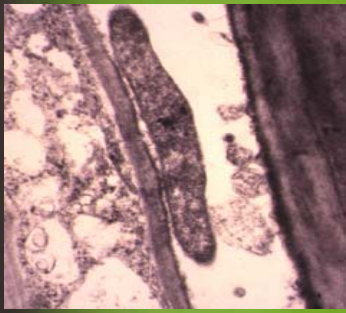
Left- Stylet of psyllid penetrating through the epidermal leaf cells and reaching the phloem tissue. The psyllid will suck out juices from the phloem cells and will ingest the greening bacteria. When feeding on another leaf on another branch or on leaves of another tree, the psyllid will inject the greening bacteria into the phloem tissue completing the infection process (Moll & Van Vuuren, 1977)



The head of a *D. citri* adult with protruding stylet ready to feed on citrus phloem.

Right -The very tip of the *Diaphorina citri* stylet showing three threads for sucking phloem sap. (Scanning Electron by Bernard Aubert)

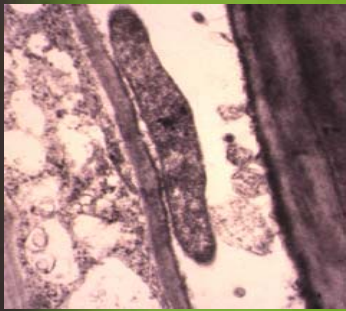




Garnier



Photo by Michael Rogers



Garnier





Survey for Citrus Huanglongbing and its Asian Citrus Psyllid in Texas

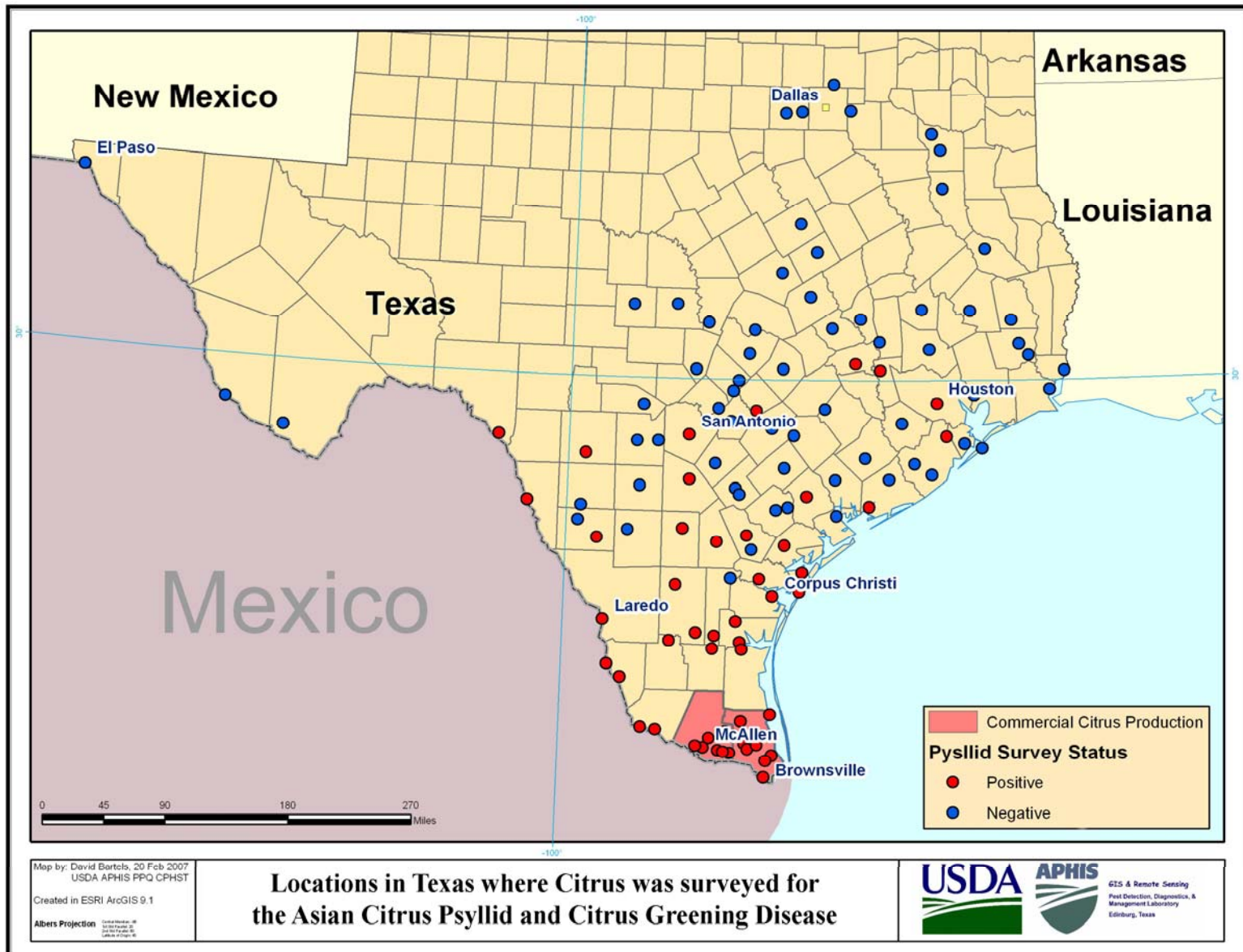
John V da Graça

J.V.French, M.Skaria, P.S.Haslem, M.Sétamou &
B.Salas

Texas A&M University-Kingsville
j-dagraca@tamuk.edu



Asian citrus psyllid *D. citri* in Texas in 2007 (courtesy da Graca et al.)



Texas Survey Summary

No. counties visited : 94

No. where citrus found : 87

Psyllids found in 32 counties

Leaf samples analyzed : 309
(all negative)

Psyllid samples analyzed : 50

2007 Survey

**800 sites surveyed – mainly
LRGV, Houston, Corpus Christi**

400 leaf samples collected

97 samples tested

All negative for greening

Asian Citrus Psyllid Detections in Mexico



July 28th 2007

- ★ Psyllid Detection
- Psyllid Positive States
- States (Mexico)
- States (United States)

Map Created By:
Jamie Perrie
GIS Specialist
USDA/APHIS/PPQ
07/26/2007
Projection: WGS 84

0 75 150 300 450 600 Miles

Epidemiology and Control

-



Death of citrus in the Reunion Islands prior to biological control of psyllids

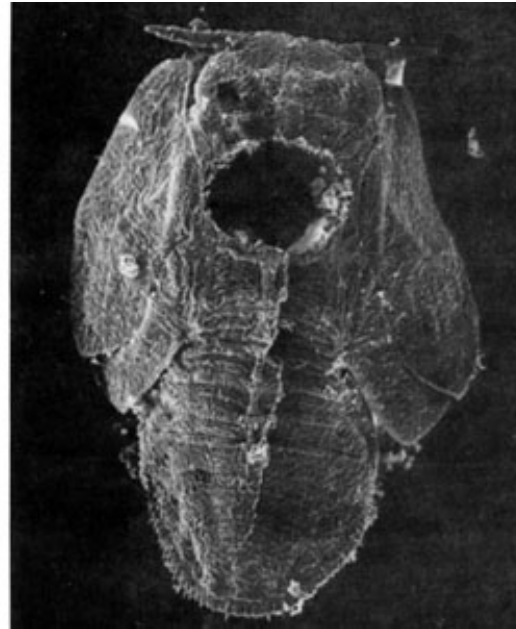
Epidemiology & Control

- **Once greening is found, the grower has to make one of the two choices**
 - **To grow citrus or Not grow citrus**
- **Intra grove management**
 - **Removal of infected trees**
 - **Vector control**
- **Regulatory support**
 - **Reduction of surrounding inoculum**
 - **Disease free planting material**
- **Early detection is the key**



Biological control of two citrus psyllids in the Reunion Islands.

- Aubert et al. showed the remarkable control of the greening disease problem in the Reunion Islands by use of disease free propagative budwood combined with release of parasitic wasps *Tamarixia dryii* and *Tamarixia radiata*.
- Shown on the left is *Trioza erytreae* parasitized with *Tamarixia dryii* and on the right, *Diaphorina citri* parasitized with *Tamarixia radiatus* ([from Aubert, 1988](#))



Letter from Christian Vernier on continued success in

Reunion - February, 2006.

- Ten year old mandarins in the Reunion Islands at Saint Benoit at 200 m altitude. In a letter from Christian Vernier:
- “Greening is still under control in Réunion due only to biological control. Furthermore, it seems that one psyllid species (the African psyllid *Trioza Erytreae*) has disappeared because the parasitoid interacted with two psyllids”



Citrus greening disease in the Philippines in 1969

- A map of the Philippine Islands taken in 1969 showing the citrus growing areas (green) and areas infected with greening disease (yellow). Much of the areas shown in yellow had gone out of production and were converted to growing sugar cane.
- Philippine mandarin production fell from 11,700 tons in 1960 to 100 tons in 1968 due to greening disease.



No tolerance to greening disease was found in 110 scion/rootstock combinations

- **In 1969 at the Citrus Experiment Station near Lipa city, 110 scion and rootstock combinations were tested for resistance to greening disease.**
- **None were found tolerant as shown in the devastation in this plot.**



A program for the control of greening disease in the Philippines by Gonzales and Viñas (1981).

PROGRAM FOR CONTROL OF GREENING

Gonzales and Viñas - Philippines

- 1. All infected trees destroyed at 50 ha station farm**
- 2. All plantings 0.5 km around station destroyed**
- 3. Greening-free nursery stock grown in greening-free area**

A program for the control of greening disease in the Philippines by Gonzales and Viñas (1981).

PROGRAM FOR CONTROL OF GREENING

Gonzales and Viñas - Philippines

4. 2,122 trees of 25 varieties planted
5. A regular 1-week to 10 day spray program
6. After 3rd year, Spray reduced to every 2-weeks
7. Continual monitoring for psyllids and yellows

- **A new planting of citrus in 1976 at the Lipa Citrus Experiment Station following a program for control of greening disease. This planting was in the same area which had been devastated by greening disease as observed in 1969.**



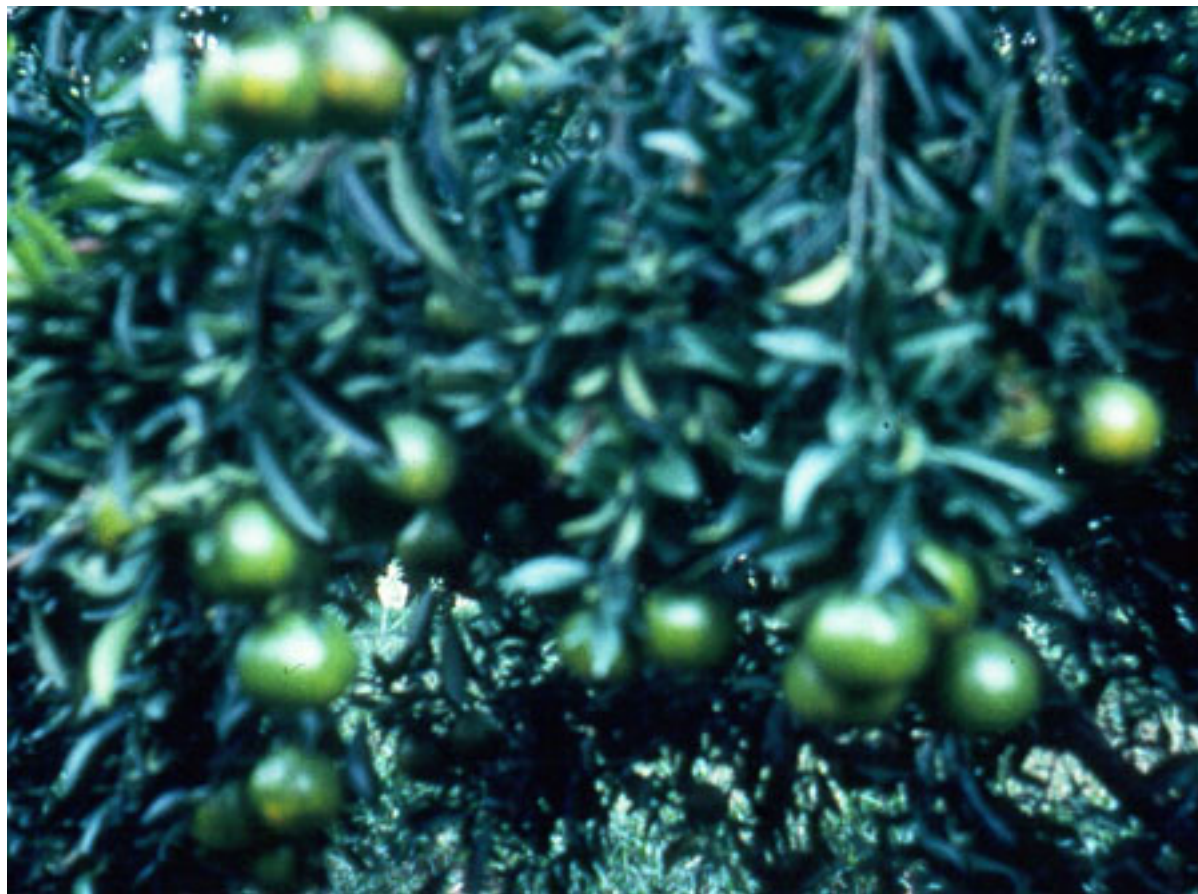
- **Shown below are trees of Ponkan mandarin at the Lipa City Citrus Experiment Station in 1981 five years after planting and following a program for control of greening disease. Note the good health of these trees in the same area which had been destroyed by greening disease (as shown on left) when observed in 1969.**



- **Showing trees of Szincom and Ladu mandarins at the Lipa Citrus Experiment Station in 1981 five years after planting and following a program for control of greening disease. Note the good health of these trees in the same area which had been destroyed by greening disease (as shown above) when observed in 1969.**



- **Showing fruit of Szincom mandarin at the Lipa Citrus Experiment Station in 1981, five years after planting and following a program for control of greening disease. Note the good size of these fruit.**



Failure of the program for greening control in the Philippines

- **This program for control of greening disease in the Philippines as developed by Gonzales and Viñas (1981) had completely broken down at the Lipa Citrus Experiment Station in the mid 1980's due to the lack of management care after those who developed the program moved away.**

Destruction of citrus in South Africa in 1977

- **Severe decline of a Valencia orange tree infected with greening disease at the Letaba Estates near Nelspruit, South Africa in 1977. Note the fruit drop and green color of fruit. Because of this classic symptom, the disease was named “greening”.**
- **Over 100,000 trees went out of production with this disease in this area during the 1970's.**



Destruction of citrus in South Africa in 1977

- **A region south of Nelspruit, South Africa where greening disease had destroyed most of the citrus in the area. In the background is a citrus packing house which had been shut down. Only a few citrus trees were to be observed in this area in 1977.**



Control strategies for greening disease in South Africa

- Use of certified pathogen tested budwood.

1. USE PATHOGEN FREE CERTIFIED TREES

Most citrus in South Africa come from
South African Citrus Improvement Program

All certified trees are grown
in a greening free area

In greening areas, all nursery trees
are grown under screen

**Control strategies for greening disease in
South Africa - Control of the vector –
Trioza erytreae.**

2. CONTROL OF PSYLLID VECTORS

**The best methods found to date
are the application of new
systemic insecticides**

By trunk application

By trunk injection

Control strategies for greening disease in South Africa

3. REDUCTION OF INOCULUM (Removal of inoculum)

Remove infected trees if under 5 years old

**Remove infected trees if 6-10 years old
if 75% or more of tree is infected**

**Remove individual infected branches
on all trees**

Letter from Dr. Hennie le Roux November, 2005

- **“We have 24 Citrus Technology Transfer groups (study groups) in southern Africa and we are constantly reminding the growers of the danger of greening disease. If the problem is neglected we shall be back to where we were within one decade.”**
- **“There is also a major difference between African greening and Asian greening. Asian greening is much more aggressive than African greening. The vector of African greening has a much narrower host range and the climate is, in most of the citrus producing areas, only suitable for the host for a couple of months of the year. If the vector is controlled in South Africa for 4 - 5 months of the year the spread of the disease is controlled. “**

Letter from Dr. Hennie le Roux November, 2005

- **“Another reason why it is easier to control the spread of the disease in South Africa is because of the Citrus Improvement Programme. At the moment 95% of all citrus trees produced within South Africa and Swaziland are produced within this scheme and we are working on legislation to clamp down on the other 5% backyard nursery trees. “**

“Lastly, I believe that in the rest of the world the citrus industries have overlooked the benefits of the stem applied systemic insecticides. The use of monocrotophos and methmidophos as stem applications are so cheap on young trees that it should be affordable up to an age of 5 - 6 years. If you give an orchard in a greening infected area that head start you have won half the battle.”

Reduction of greening disease in China

- Ke Chung and Xu Chang Fan ([1990](#)) reported profitable growth of citrus in greening affected areas of China by good management practices. The program they followed is very similar to that of the Philippine program.

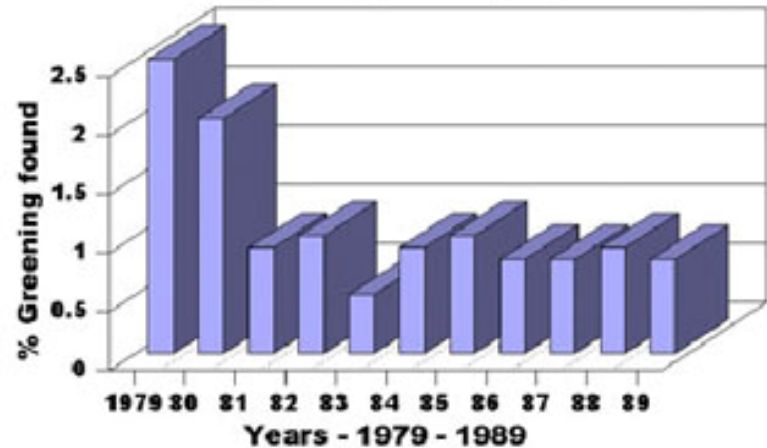
GREENING CONTROL STRATEGIES

1. PLANT CERTIFIED TREES ONLY
2. MONITOR FOR PSYLLID VECTORS
3. TIMELY SPRAY APPLICATION
4. REMOVE ALL INFECTED TREES AND BRANCHES
5. EDUCATIONAL PROGRAMS

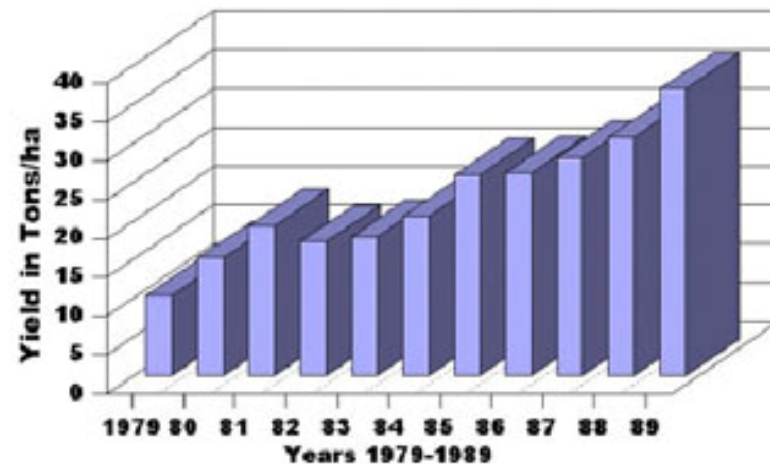
Reduction of greening at the Red Star farm.

- (above) Following a program for control of greening disease at the Red Star farm at Fuzhou, China, the disease was reduced from 2.5% to less than 1% over a 10 year period from 1979-1989. These control strategies were applied to an existing greening-infested farm.
- Shown below is the corresponding increase in yield from 10 T/ha to 37 T/ha over this 10 year period. Data taken from Ke Chung and Xu Chang Fan (1990).

Reduction of greening - Red Star Farm - China
With a greening control program

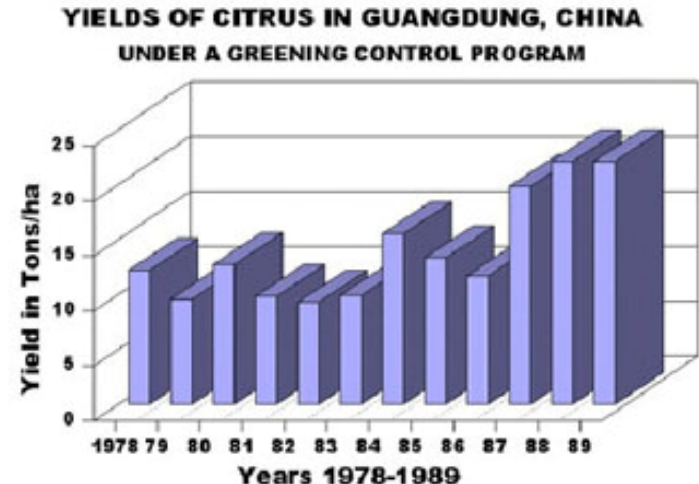
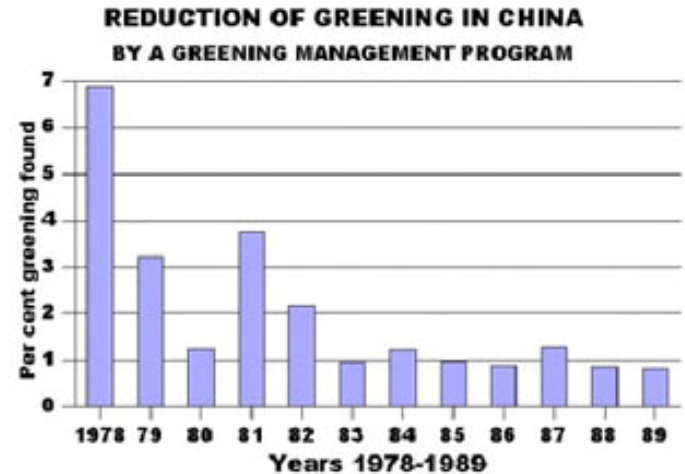


Yield of citrus in Fuzhou, China
With a greening management program



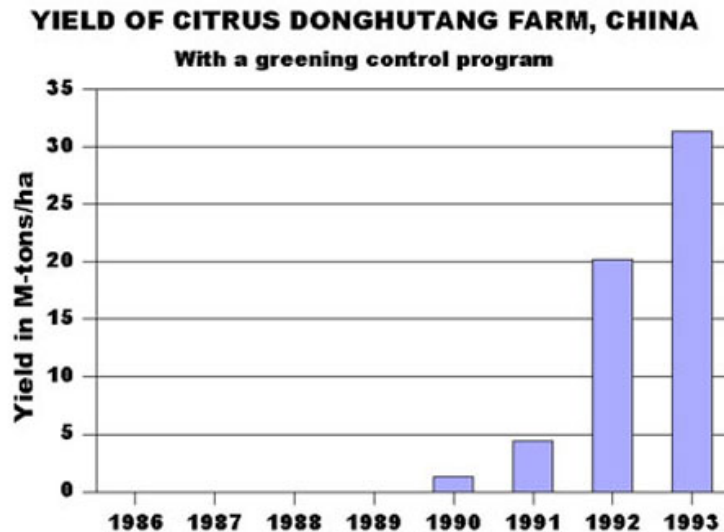
Reduction of greening at the Yancun farm.

- A graph showing the reduction of greening at the Yancun farm, Boluo county in central Guangdong Province, China over an 11 year period from 1978-1989.
- The Yancun citrus farm was one of the largest citrus farms in China, Greening disease was reduced from 7% in 1978 to less than 1% in just a few years.
- Data taken from Ke Chung and Xu Chang Fan ([1990](#)).



Excellent results can be obtained if certified greening-free plants are used and proper control practiced applied.

- A graph showing what can be accomplished if you start with clean budwood in a new grove and follow the practiced for good management control of greening. A new planting of trees in 1986 gave an ever increasing yield to 32 T/ha in 1994. Greening control strategies can work if control strategies are rigorously practiced and maintained.



However – this fine program failed!

A letter from J.M. Bove - November, 2005

- “Regarding the situation at the "Oversea Chinese farm at Yangcun town, Boluo County, Guangdong province, I remembered it very well, having seen it in 1987 and 1995. Here is what I was told from Prof. Liu Lihua, Fuzhou: "As I know, the former huaquiao farm has been changed to the agricultural institute and they only produce a little of citrus seedlings, and there is no citrus orchards in this area". As a matter of fact, I learned that the farm went down with HLB, essentially because of mismanagement, change of director, and the use of miticides instead of insecticides to control the psyllids.
- The decline of this farm illustrates the fact that in China a citrus orchard goes down with HLB within 5 to 6 years if the following two measures are not fulfilled to control HLB: 1) frequent surveys to spot symptomatic trees and immediate removal of the spotted trees, 2) excellent insecticide control of the psyllids. ”

Devastation of citrus by greening disease near Phrae, Thailand in 1994

- Showing citrus in total decline due to greening disease.
- Many citrus areas in Thailand had gone out of production as unprofitable where trees lived for only 6 to 7 years.



Devastation of citrus by greening disease near Phrae, Thailand in 1994

- **Another grove of citrus observed near Phrae, Thailand in 1994. These 6 to 7 year old trees were in complete decline due to greening disease.**



The importance of education

- C.N. Roistacher talking with growers in the region near Phrae, Thailand in 1994. Based on this experience, it was decided to produce a comic book which would explain the problems and solutions which the growers could understand to help them in their fight against this serious disease.



A comic book in the Thai language on greening disease.

- The opening page of this comic book.
- The translation reads “Profit (above) or Loss (below).”



A comic book in the Thai language on greening disease.

- In the second page of the comic book the farmer asks the extension worker: “I would like to know why my tangerine trees are dying and what can I do about it? I feel it is costing me money and my profits are so little”.

The extension worker answers “Perhaps I can be of help”.



A comic book in the Thai language on greening disease.



ผมเข้าใจว่าต้นส้มของคุณที่กำลังจะตาย
คงเป็นโรคที่เกิดจากเชื้อแบคทีเรีย



ภาพแสดงตัวแก่ ไข่และตัวอ่อน ของเพลี้ยไก่แจ้

ต้นส้มของคุณติดโรค ได้เช่นเดียวกับที่
คนติดเชื้อมาแล้ว



A comic book in the Thai language on greening disease.



The economics of growing citrus in Thailand.

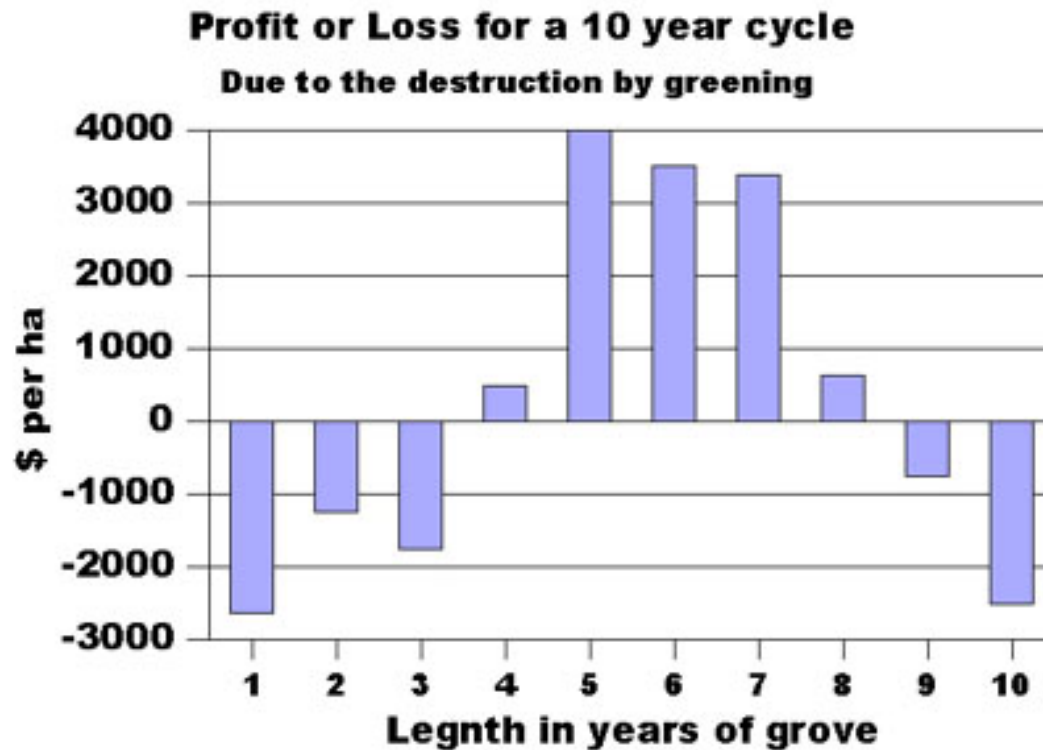
Citrus in Thailand live for an average of 5 to 10 years due to the greening disease

- If trees live for 6 years the loss is about \$1,400/ha and citrus would go out of production.
- If trees live for 8 years the loss would be about \$300/ha. This was the general situation observed in Thailand in 1994.



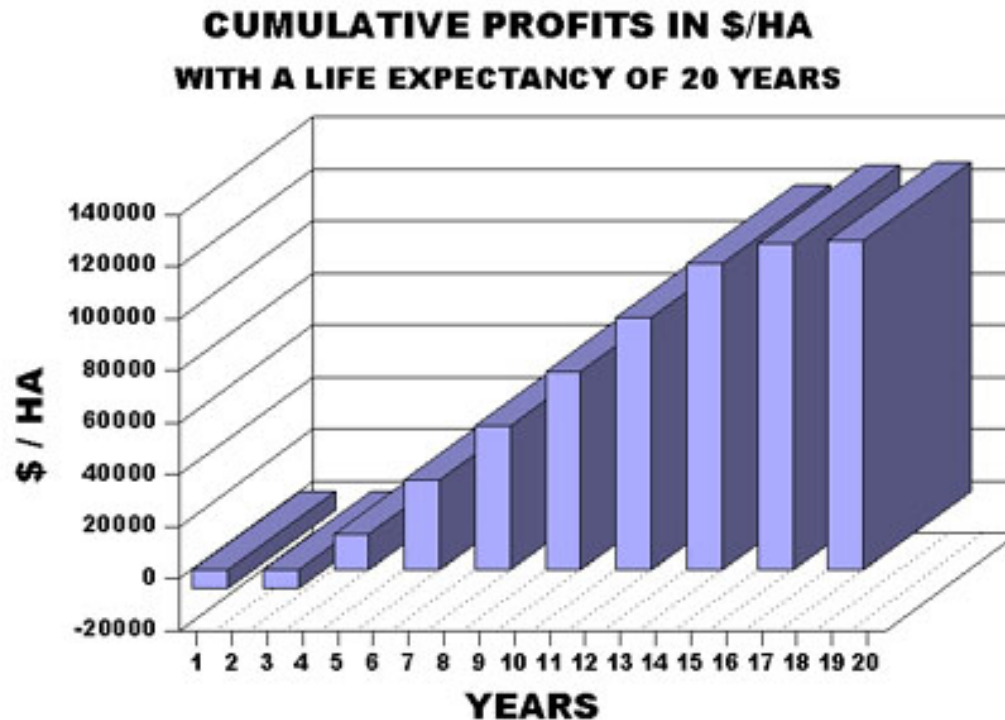
The yields and returns of citrus trees which live for 10 years.

- If trees live for 10 years a grower would just barely make a slight profit.
- These are from studies of Grenzebach, [\(1994\)](#).



The potential profit if trees could be made to live for 20 years in Thailand.

- It was difficult for growers in Thailand to realize that a citrus tree could live for over a 100 years. They were amazed when they saw a picture of the Parent navel Washington orange tree 120 years old



The program for control of greening disease in Thailand has failed due to a lack of an aggressive education program. If trees live for an average of 8 years growers realize a small profit and live with this.

- **Below, showing two year old trees of Som-Kiawan mandarin at the experiment station at Chiang Rai, Thailand. All trees in this plot were originally virus tested and free of greening disease when planted. It is evident that greening came into this plot from the adjoining field which, like most citrus in Thailand is heavily infected, Infection in this plot occurred within two years.**



**Why did greening control fail in
Thailand?**

or

**In almost all countries in the world where
greening disease is endemic?**

Greening in India

- **Shown here in 1992 is a severely affected tree of Coorg mandarin in a grove near Gonicopol.**
- **Dr. Manjuneth, who worked in Gonikopal in 1992 writes that “When I went back in 1996, it was very difficult to find citrus trees there.”**



Greening in India

- **Greening on sweet orange at the Khosha farm near New Dehli, India in 1992. Temperatures were reported as high as 46C for two to three months during the summer and can reach as high as 50C. Warm temperatures favor decline of citrus with the Asian greening disease.**



Greening in India

- Dying sweet orange trees with greening disease at Hindupur near Bangalore.



Greening in India – Contrast in sensitivity of lime and sweet orange.

- In a citrus experimental orchard near Hindespoor, India in 1992, a row of sweet orange trees (right) was next to a row of small fruited lime trees.
-
- Shown is the contrast in sensitivity of the two citrus varieties. The sweet orange trees were mostly all dead while the lime trees (left) were in full leaf. However, as shown in the inset, mild but classic mottle was evident on many of the lime leaves.



Greening in India - 100% infected. No effective control program in the country

- A field of dead trees at the Indian Horticultural Research Station at Mysore observed in 1992. The extensive variety collection was 100% infected with greening disease. Almost every scion and rootstock was severely infected.
- There is no effective control program in the country and trees live for 5 to 12 years. Warm temperatures favor rapid spread and decline of trees.



Severe problem of greening in Indonesia

- **An epidemic of greening disease occurred in Indonesia in the early 1960's resulting in an average productivity of less than 8t/ha.**
- **A massive eradication program was undertaken with the destruction of over 3.6 million trees in the province of Bali in the hopes of reviving the industry. This represented over 70% of the citrus in Bali.**
- **Over 1.6 million trees from 120 shoot tip grafted sources were planted in 1991 with an estimated production of 12 million trees by 1994. It was expected that with eradication and planting greening-free trees, the industry would be revived.**
- **By July, 1994, 39% of the newly planted mandarin trees showed symptoms of greening disease and by May, 1995 the percentage had reached 76%.**
- **Today (2007) citrus cannot be profitably grown in Indonesia.**

GREENING IN FLORIDA – The initial discovery of greening disease on a pummelo tree near Homestead, Florida in August, 2005.

- From the Florida Citrus Industry Magazine, Nov.-Dec. 2005. “It took just two months after finding the first case of citrus greening in Florida for State and Federal agriculture officials to conclude that **eradication of that bacterial disease is impossible.**”



The first outbreak of greening in Florida

Symptoms of greening found on sweet orange in Florida.

- A greening infected tree of sweet orange with characteristic leaf mottle and fruit with stylar end greening.
- Note the asymmetric leaf mottle)



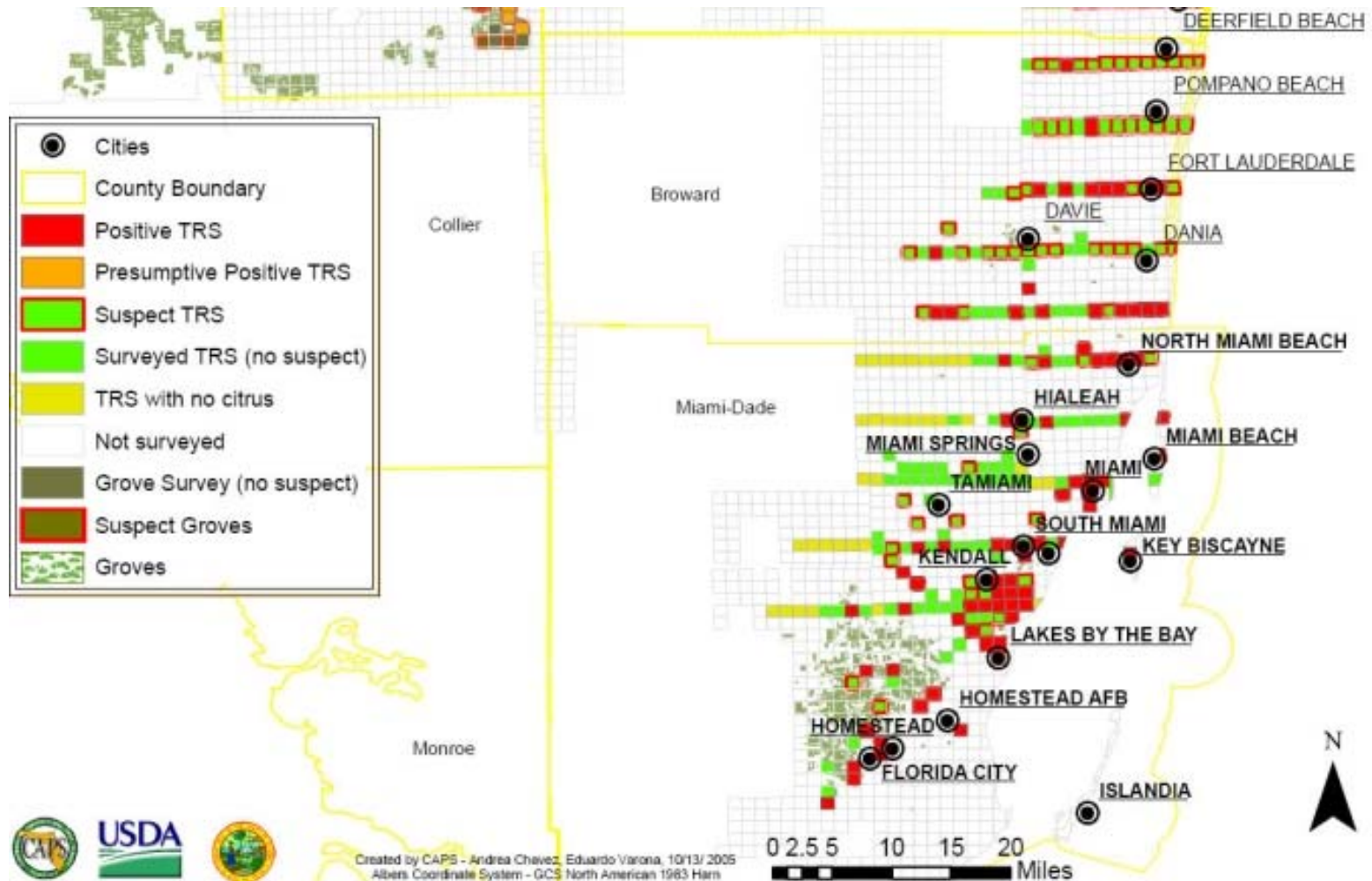
Photos J.M. Bove, Nov. 2005

A greening affected tree found growing in a side yard in Palmetto Bay, near Miami in Dade County. Showing severe yellowing and leaf mottle symptoms.



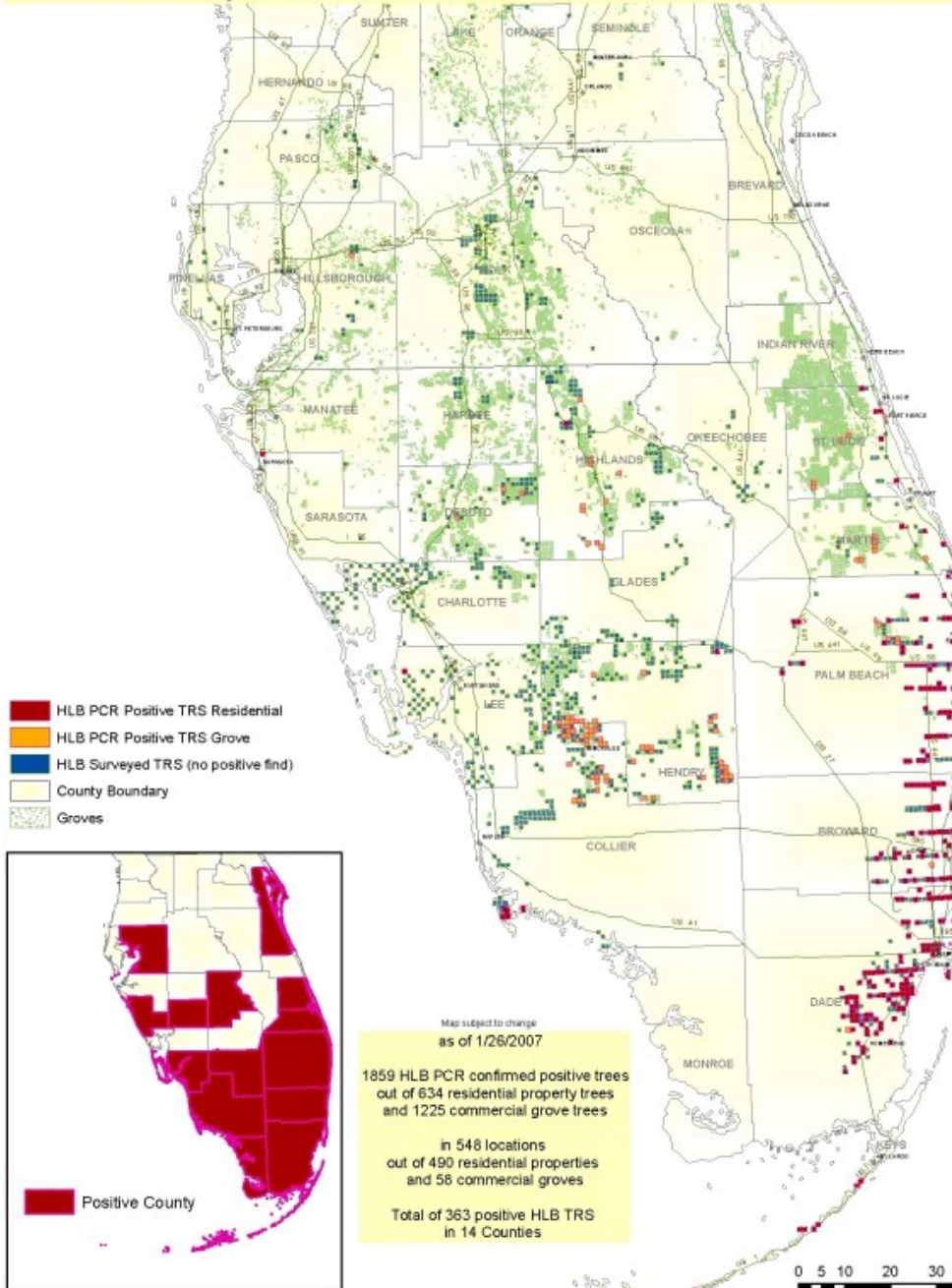
Survey for greening disease (huanglongbing) six weeks after the initial discovery

- Photo taken October 13, 2005 courtesy Florida Department of Agriculture and Consumer Services.



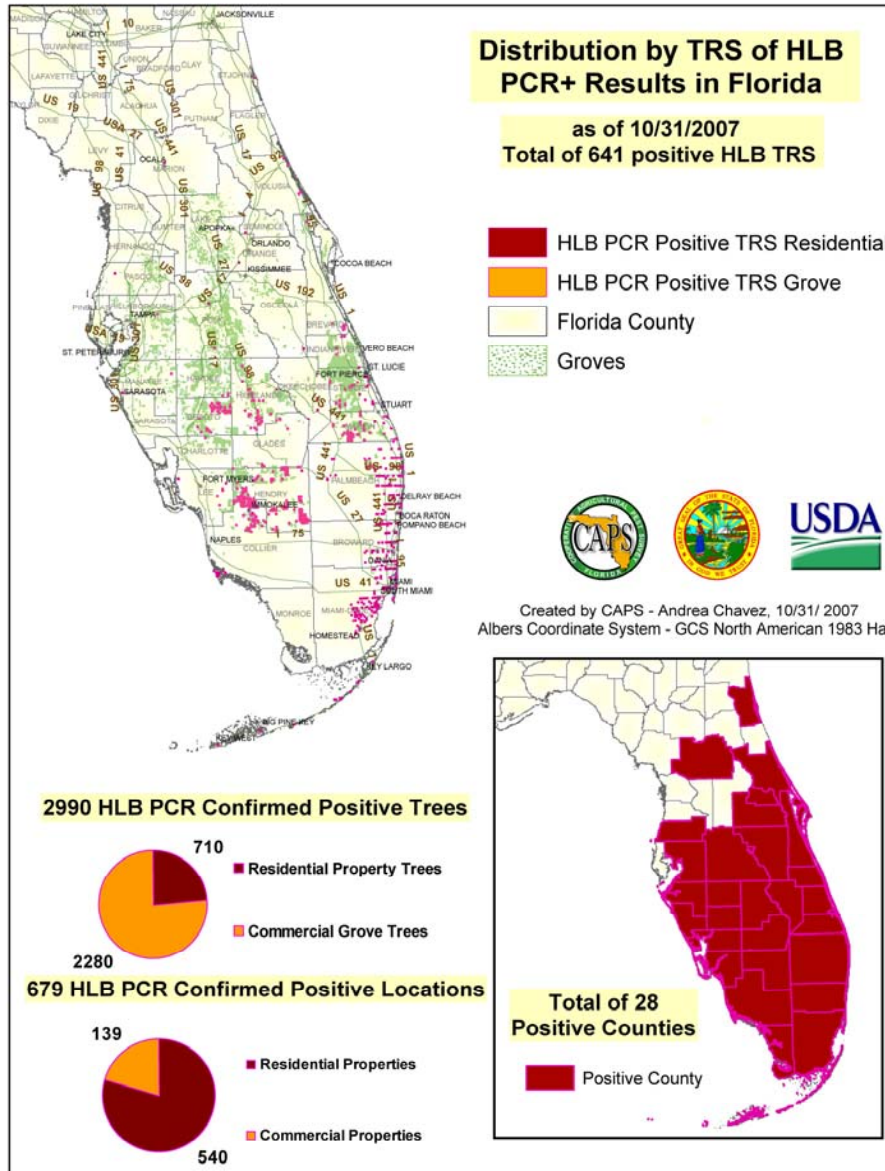
Greening spread In Florida January, 2007

Situational Map of HLB PCR Results - Commercial and Residential



Extent of areenina in Florida October 31, 2007

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HUANGLONGBING IN BRAZIL

IMPORTANCE

GEOGRAPHIC DISTRIBUTION

CONTROL

I thank Juliano Ayres for this set of slides
Presented at the 17th conference of IOCV
Adana, Turkey, November 2007

Antônio Juliano Ayres

Fundecitrus

INCIDENCE EVOLUTION

Affected Grove

Affected Grove

Westin

July/2004 (0,3%)

11 months

Affected Grove

Affected Grove

May/2005 (15%)

Neighbor's Effect



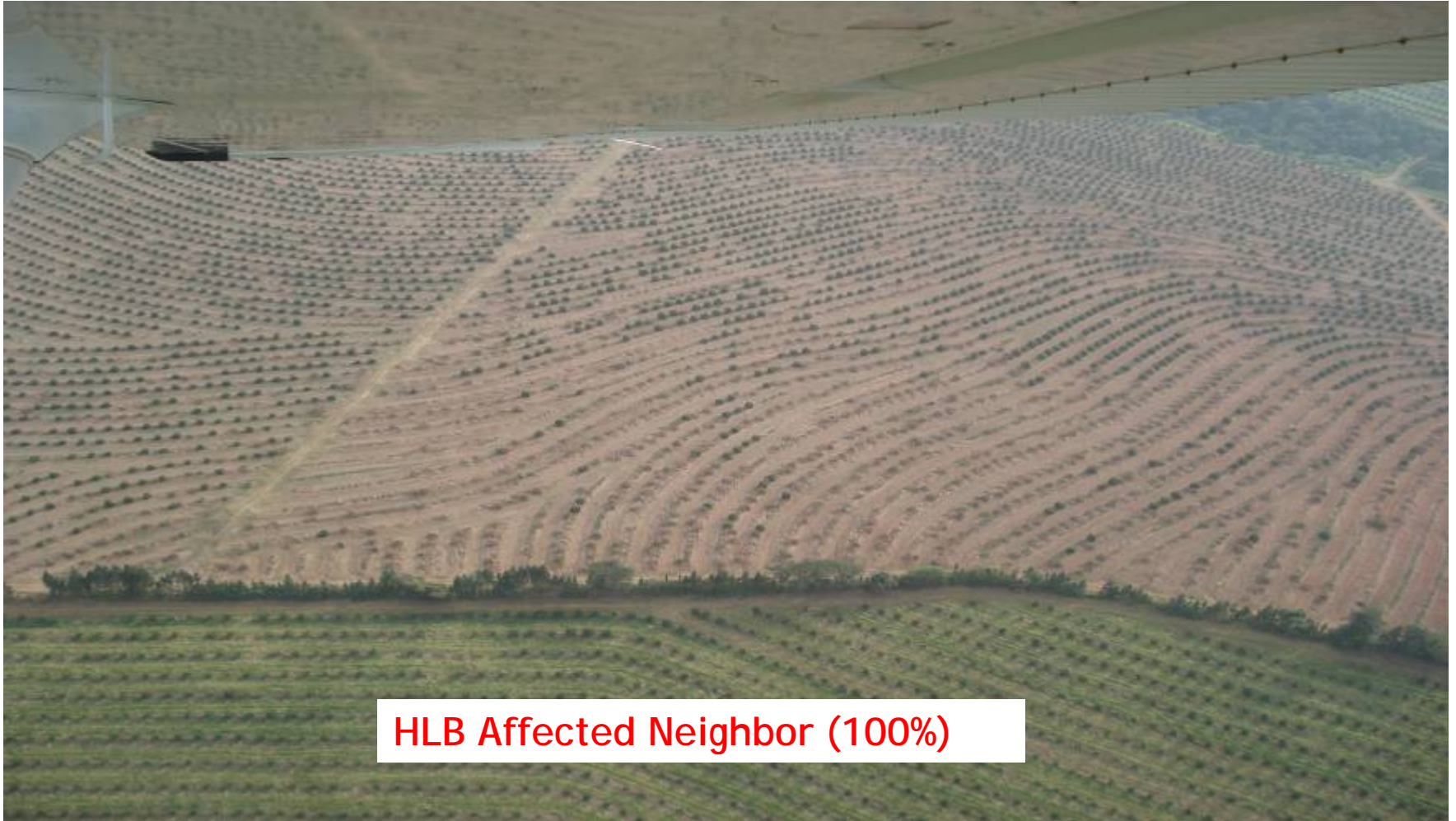
> 40% HLB

The image is an aerial photograph of agricultural fields. A red line runs diagonally across the middle of the frame, separating a healthier, greener area in the lower-left from a more brown and sparse area in the upper-right. The text is overlaid on the image.

100% HLB

Negligent Neighbor

Neighbor's Effect

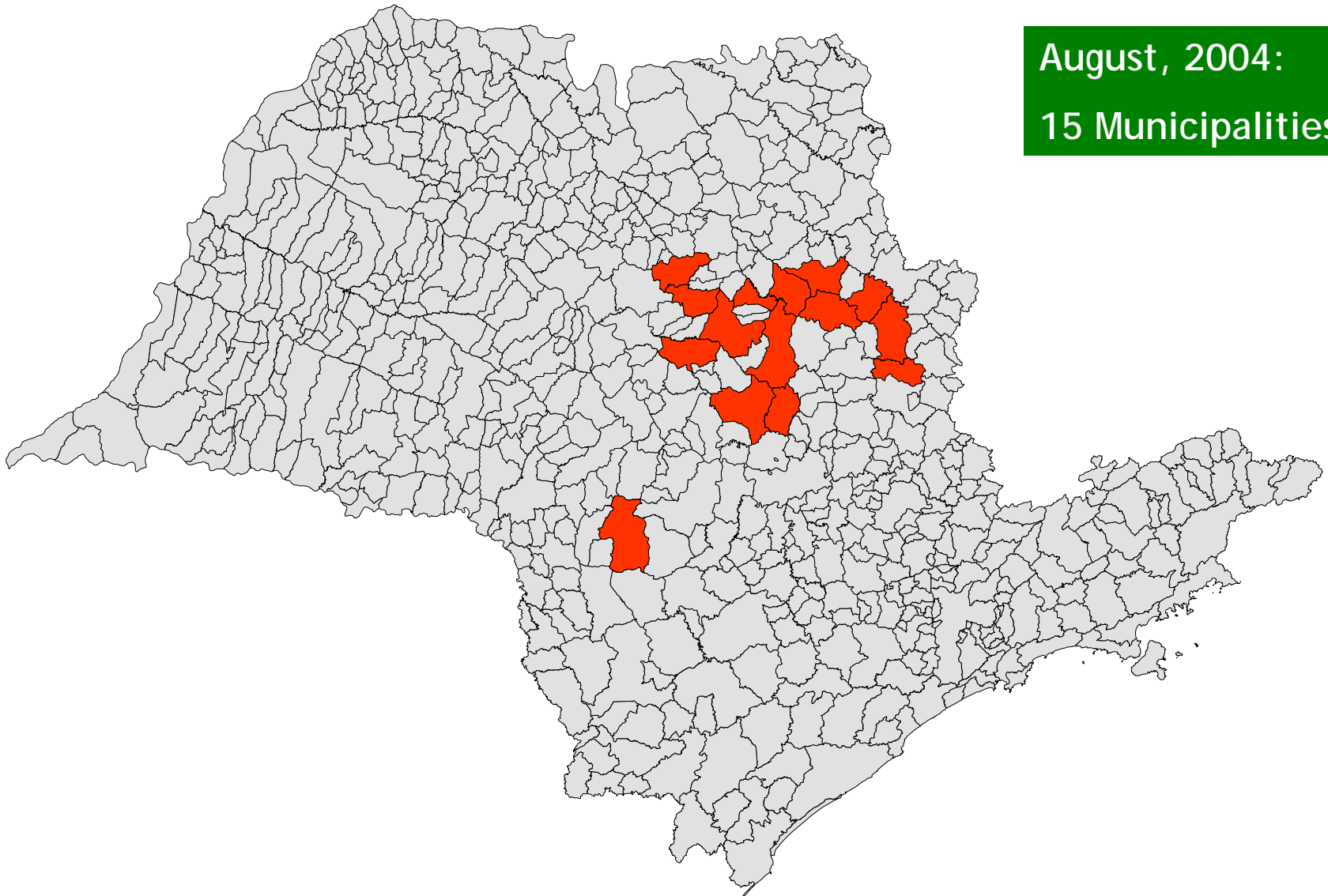


HLB Affected Neighbor (100%)



Distribution in São Paulo State

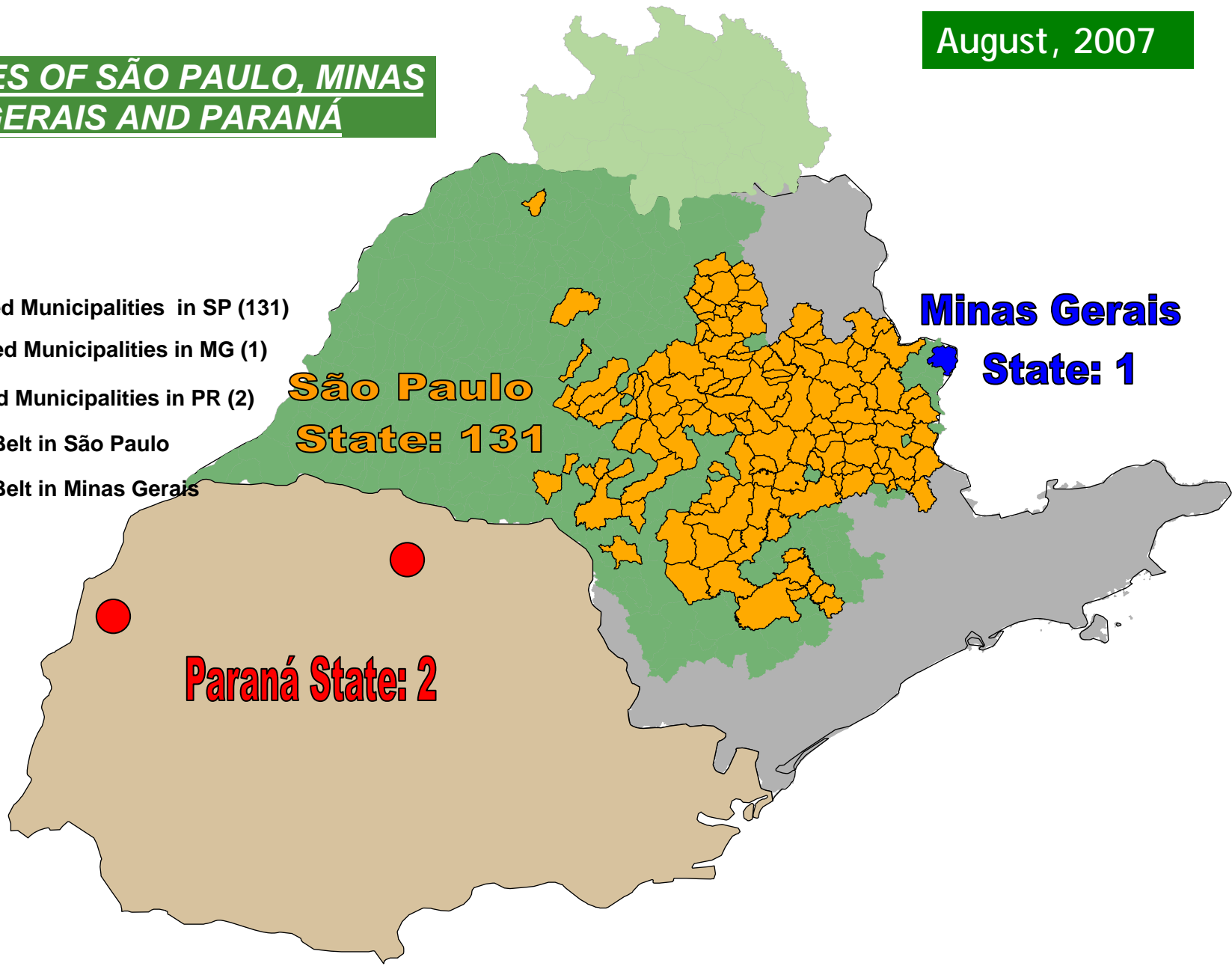
August, 2004:
15 Municipalities



August, 2007

**STATES OF SÃO PAULO, MINAS
GERAIS AND PARANÁ**

-  Affected Municipalities in SP (131)
-  Affected Municipalities in MG (1)
-  Affected Municipalities in PR (2)
-  Citrus Belt in São Paulo
-  Citrus Belt in Minas Gerais



CONTROL

1. Inspection and elimination of symptomatic trees



**HLB
Control**



**2. Chemical control of
psyllids**

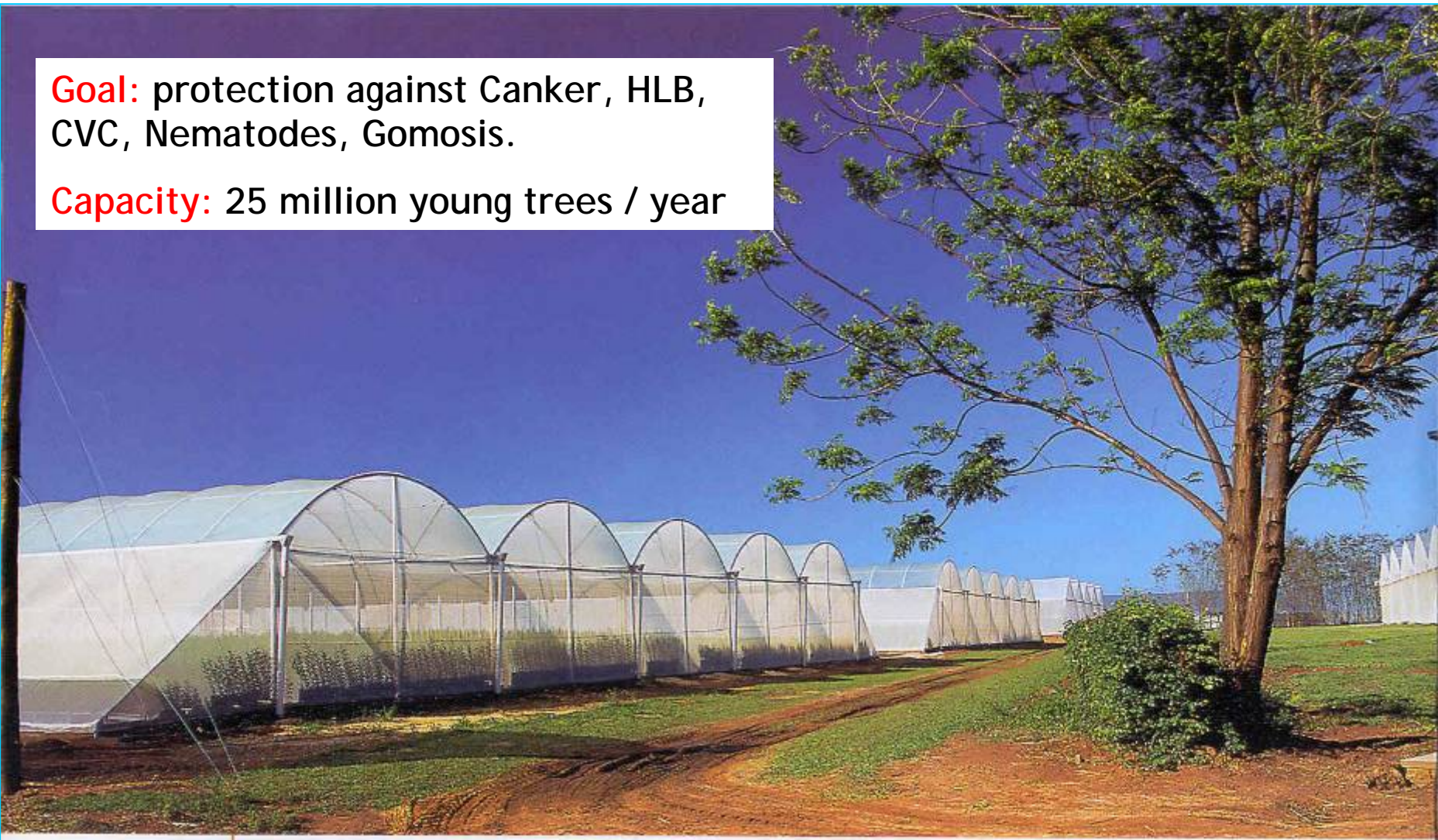


3. Healthy young plant

NURSERY: MANDATORY PROGRAM

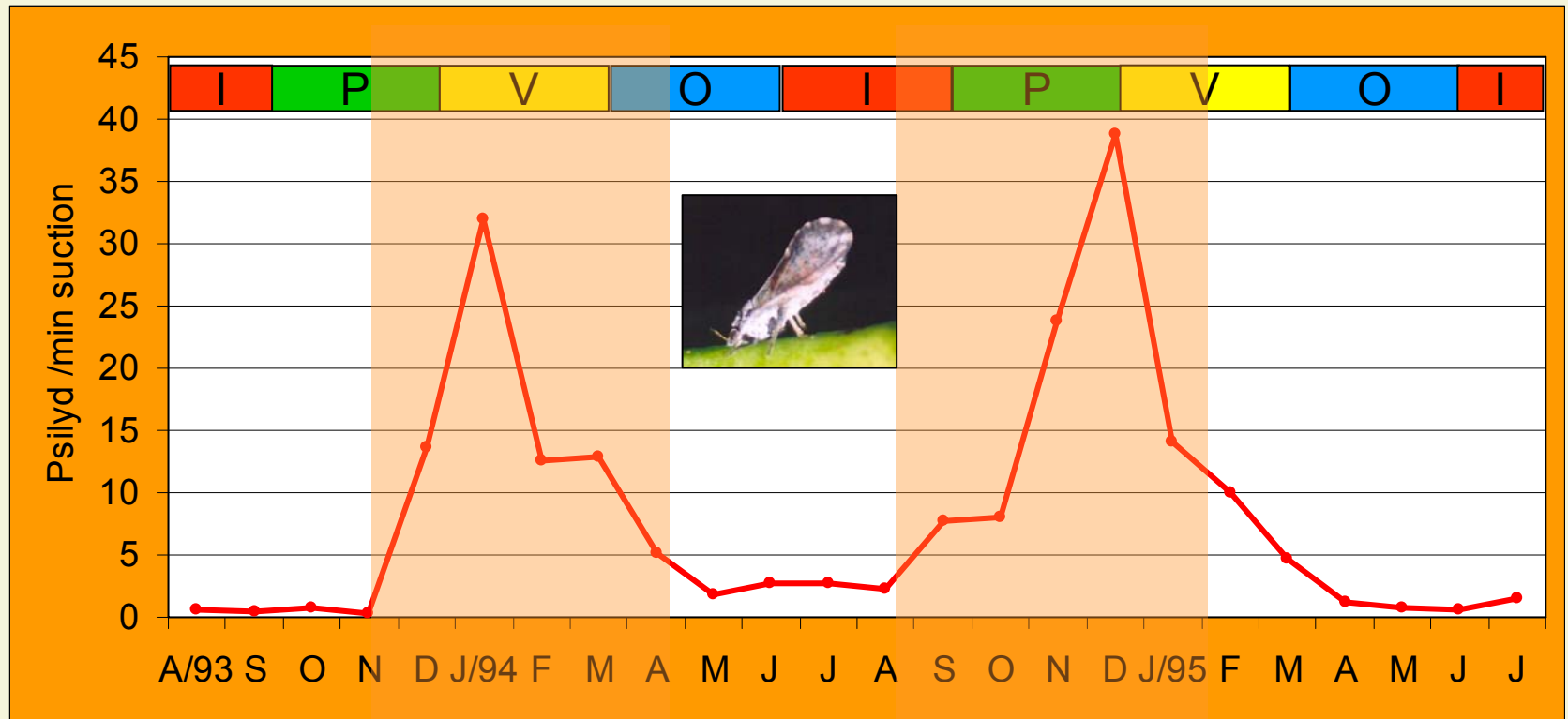
Goal: protection against Canker, HLB, CVC, Nematodes, Gomosis.

Capacity: 25 million young trees / year



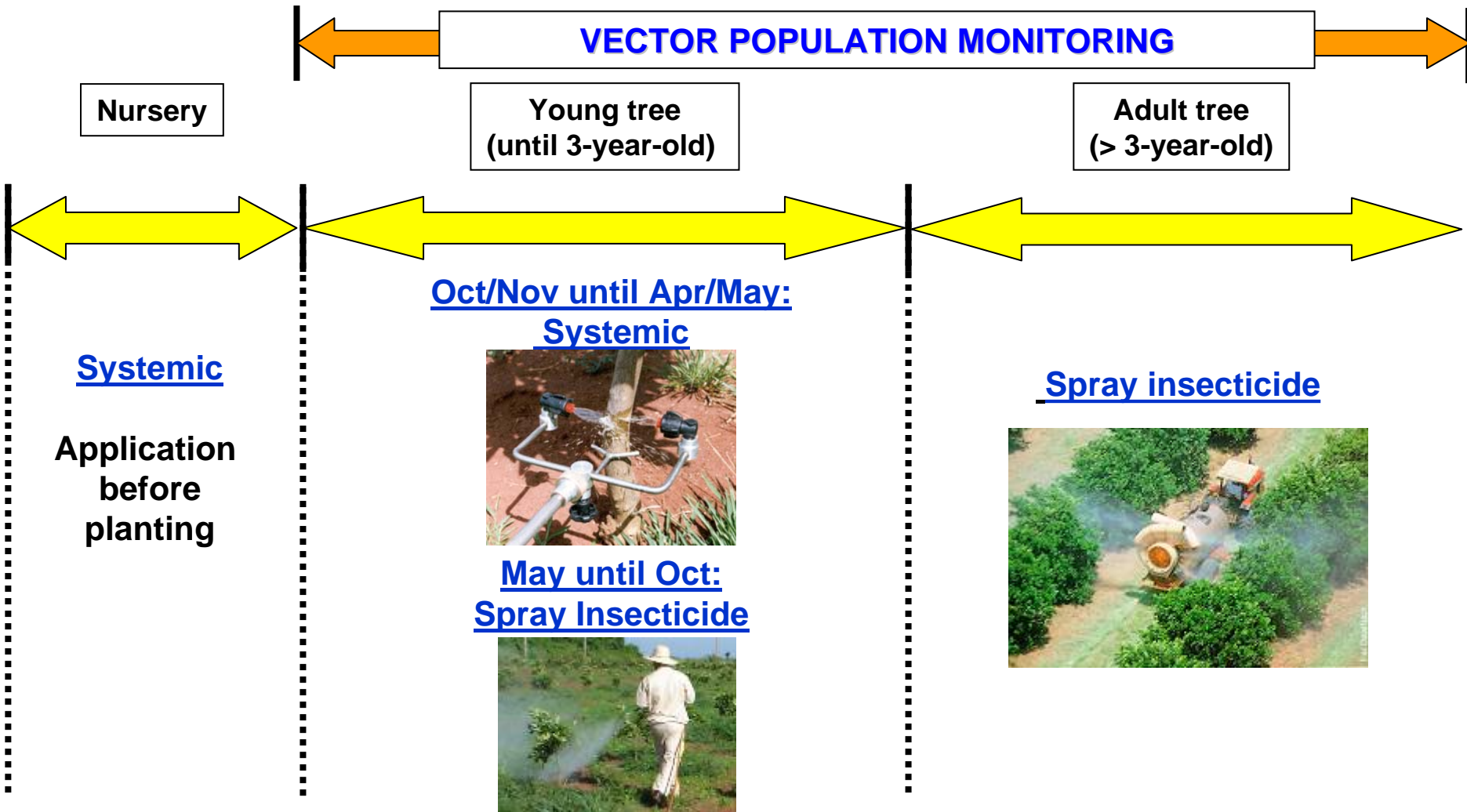
VECTOR AND CONTROL

Diaphorina citri – Population Dynamics



Population Averages in the North of São Paulo State (1993-1995)

- Chemical control of sharpshooters and psylla - Suggestion of insecticide application



Airplane Insecticide Application



INSPECTION

Inspections

Ground



Top Platform



PLATFORM INSPECTION



Inspection : Platform, Carefully, Many times/year



Results of shoot pruning



5 months later



Lopes et al. 2007

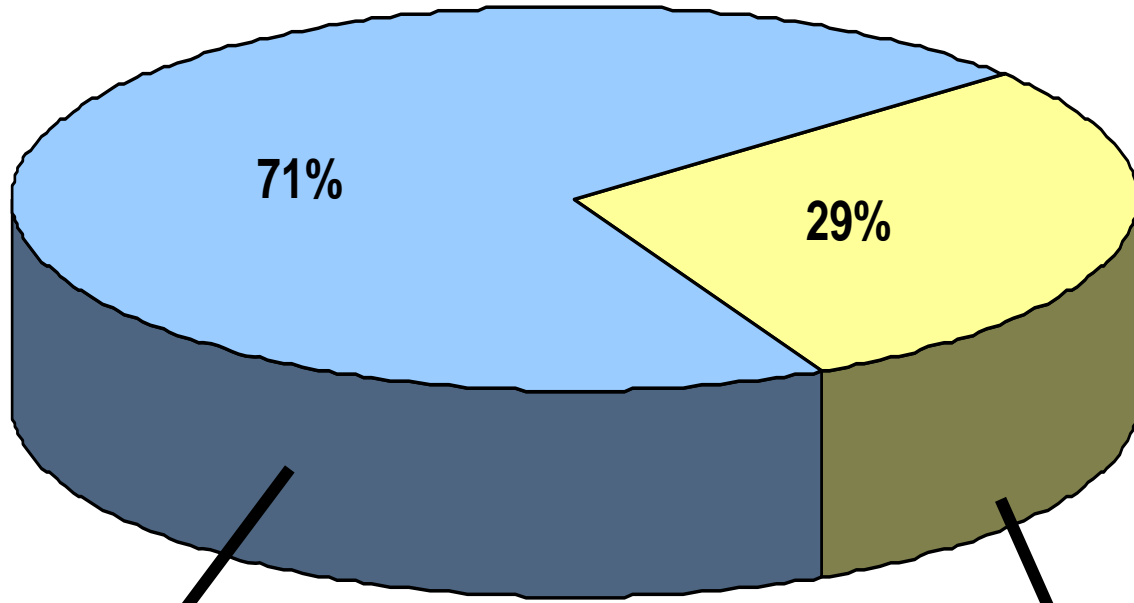
HLB Tree Elimination



Root
Elimination



Positive Samples



Groves bordering affected neighbors

Groves bordering non affected neighbors

CONTROL PROGRAM STRATEGIES

1. Increase the consciousness of the grower

Goal: Voluntary elimination of symptomatic plants

2. Federal law - Enforcement

Goal: Mandatory elimination of symptomatic plants

STRATEGIES TO CALL GROWER'S ATTENTION

Communication campaign

- . TVs, Radios, Magazines, Manuals, Cards, etc...

International seminars (2)

- . 1,000 people

Lectures/ Speeches


- . 12,000 citrus farmers

Inspector training

- . 5,200 people from farms



STATUS OF THE CONTROL PROGRAM

 2.0 million symptomatic trees
eliminated voluntarily by the growers until July, 2007



FEDERAL LAW

- 1- Inspection by the grower
- 2- Elimination of citrus trees showing HLB symptoms and *Murraya paniculata* trees
- 3- Production of healthy young plants in screenhouses

CHALLENGES

- 1. Citrus grower must be convinced that frequent re-inspection and vector control are essential**
- 2. Citrus grower must work together to succeed against HLB**

Conclusions

- **Once Asian greening enters a country, it is extremely difficult to control**
- **We have shown failure to control the spread of Asian greening in many countries i.e. Philippine Islands, Thailand, Indonesia, India, China, and Florida.**
- **Florida may be on the verge of losing its citrus industry.**
- **Brazil is now undergoing a life and death struggle to preserve its industry from the ravages of greening disease.**
- **Texas has initiated new laws and is looking hard for the greening organism**
- **The *Diaphorina citri* is now well established in Mexico and countries of the Caribbean region.**
- **What needs to be done in these countries to prevent the outbreak of greening disease?**

Diaphorina citri in Mexico – 2007

There is a need for Emergency surveying of citrus in Mexico!



Mexico and all of the countries of the Caribbean region where *Diaphorina citri* is now present are like dry forests waiting for a match to ignite the firestorm of greening disease – not too different from the firestorm that struck California in October of 2007



As currently in Texas, a program is needed to search for the presence of greening-infected trees in your country. Use all means to detect the greening organism. Constant vigilance is needed to prevent the disasters as shown in this presentation!





Thank you!