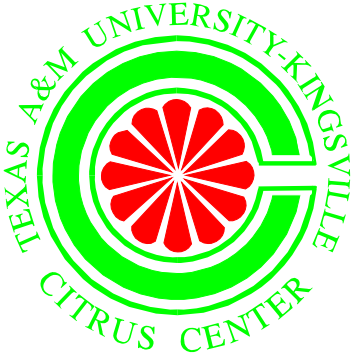
History, Etiology and Worldwide Situation of Huanglongbing

J. V. da Graça



Greening/HLB

What's in a Name?

- Huanglongbing (Yellow shoot disease) – China
- Greening or Yellow branch (South Africa)
- Likubin (drooping disease) – Taiwan
- Mottle leaf – Philippines
- Citrus die-back (India)
- Phloem degeneration (Indonesia)

Is HLB the same as Greening?

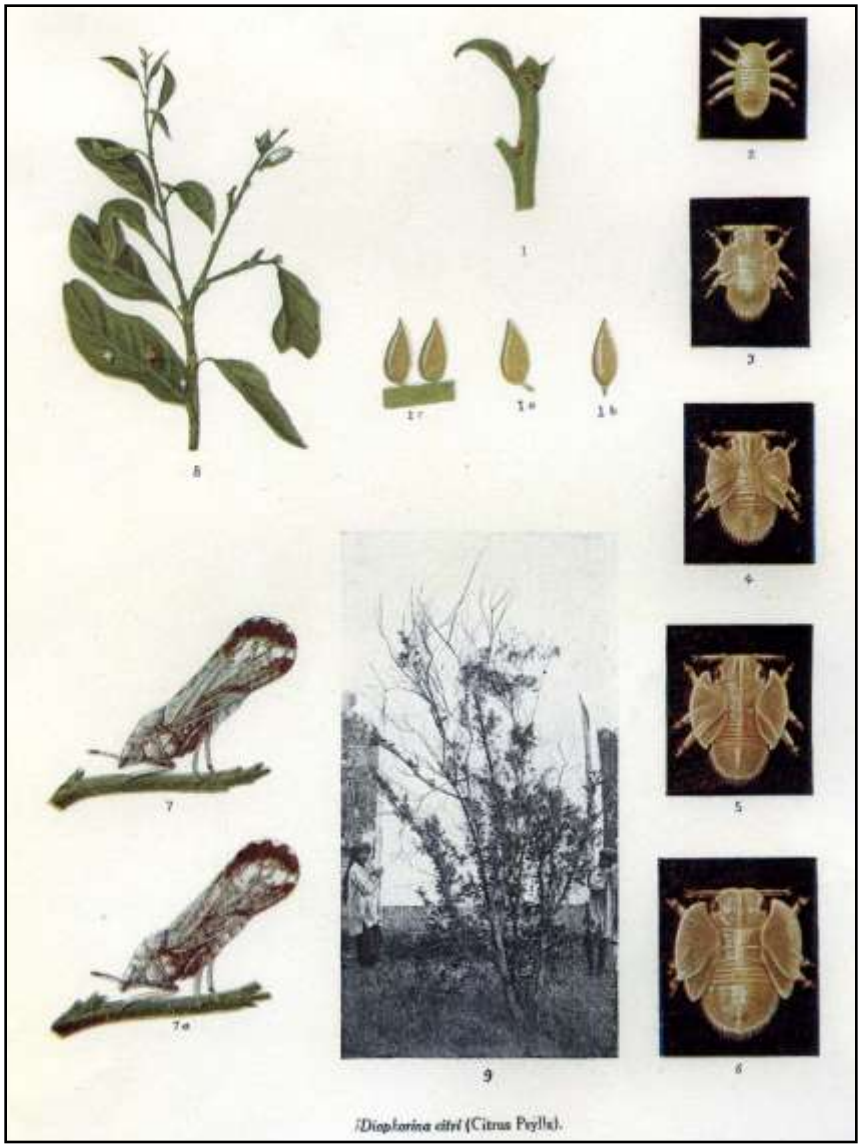
Yellow shoot = Yellow branch

History

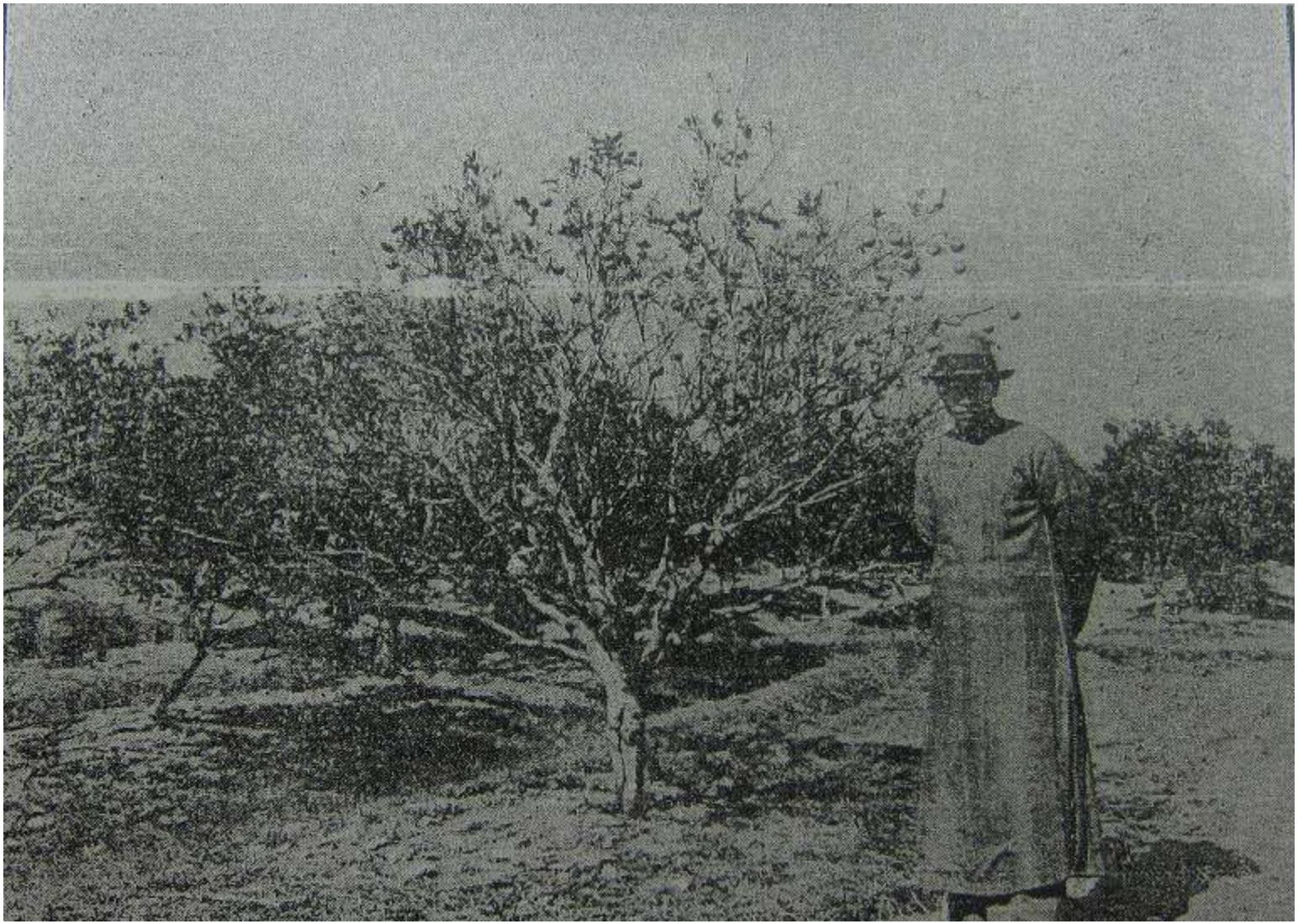
- Origins uncertain.
- 1. Was “Citrus Die-Back” in India in 18th century HLB?
- 2. Was “Yellow Shoot” in southern China in late 1800s HLB?
- 3. Was “Mottle Leaf” in Philippines in 1921 HLB?
- 1. Maybe; 2. & 3. Probably not.

Where did HLB originate?

- First clear description of the symptoms of HLB by Husain & Nath (1927) in India/Pakistan.
- Describes *Diaphorina citri* 'damage':
- *"The only sign of injury is defoliation and death of the shoots attacked and the drying up of the branches."*
- *"the fruit of the infested tree is dry and insipid to taste"*



Husain & Nath (1927)



Citrus HLB in Guangdong in 1930’S
First published description 1938

History of HLB/Greening in S Africa

- 1928 farmers near Pretoria found citrus trees with symptoms: ‘Yellow branch’
- 1929 ‘Greening’ reported in E. Transvaal (now Mpumalanga)
- 1937 – suggested YB may be Cr toxicity and Greening Mn deficiency
- 1933-44 reported to spread through E. Transvaal
- Amongst suggested causes - virus

“South African Cr/Mn toxicity”

- Van der Merwe & Anderssen (1937)
- *“The fruits are greenish in colour, usually on one side only, small, often misshapen, usually seedless and of poor quality” “Usually such fruit readily drop…”*
- *“..leaves on one or two branches only of a tree turn yellow.” “…mottle leaf…”*

Chromium and Manganese Toxicity.

Is it Important in Transvaal Citrus Growing?

By A. J. v. d. Merwe, Research Horticulturist, and Dr. F. G. Andersson, Chief Horticulturist, Division of Plant Industry

FOR a number of years two serious diseases have been known to citrus growers in the Transvaal. The one disease occurring in the western Transvaal is often referred to as "yellow branch", and the other, in the eastern Transvaal, is locally referred to as "greening" or "arrested development". These two maladies are very serious in some orchards and are reported to cause crop losses amounting to as much as 25 per cent. With the progressive development of the disease, whole orchards have been completely wiped out.

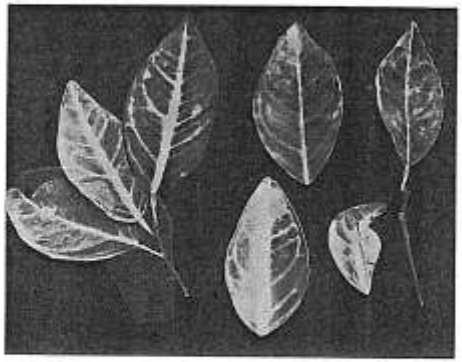


Fig. 1.—Yellowing on the veins of citrus leaves.

Description of Malady.

The local names for these diseases are partially self-explanatory. As the malady in the western Transvaal is probably different from that in the eastern Transvaal, even though they appear to be very similar in some respects, the symptoms will be described separately.

"Yellow Branch."—This trouble first manifests itself by leaves on one or two branches only of a tree turning yellow. The yellowing usually starts on the veins while the sections between the veins remain green. This condition is shown in Fig. 1. Often, however, it is the area between the veins which turns yellow while the veins remain green; such a condition is very similar in appearance to the so-called "mottle leaf" which has often been described in literature and which is cured by applications of zinc; leaves affected in this way are illustrated in Fig. 2.

In acute cases of this yellowing, the whole leaf turns yellow. Old leaves seem to be more seriously affected than are new ones.

In all cases, the yellowing or mottling of the leaves is followed by dying-back of the tips of the wood, and eventually the whole tree may die. In the case of badly affected trees, the bark becomes loose and scaly, and the roots die back.

The fruits from such "yellowed" branches or trees are entirely unsuitable for commercial purposes. They are greenish in colour, usually on one side only, small, often misshapen, usually seedless and of poor quality. Their external appearance is such that they can usually be distinguished easily from normal fruit. Usually, such fruit readily drop from the tree.

"Greening."—The leaf and twig symptoms of this disease, which is prevalent in the eastern Transvaal, are nearly identical with those often described as "mottle leaf" and are illustrated in Fig. 2. Usually it also appears on some branches before appearing on others of the same tree.

The appearance of the fruit is very similar to that described for "yellow branch".

In neither of the above cases does the application of zinc ensure a marked recovery, although some temporary beneficial effect has been obtained in a few cases of "greening".

In Plants other than Citrus?

In avocadoes a yellowing in the form of blotches on the leaves of certain branches has been noted, the branches dying back. Such trees are growing in the same environment as that in which "yellow branch" is prevalent in citrus, and it is most likely that the cause is the same in both cases.

In the case of gardenia plants, a chlorotic condition, identical in appearance to lime-induced chlorosis, has been noted. The soil conditions are such, however, that lime-induced chlorosis is impossible, and it is suspected that the trouble is due to the same cause as "yellow branch" in citrus. Dead roots and scaly bark have also been noticed, as in the case of citrus.

Tobacco, growing in the same area where "yellow branch" is serious in citrus, has been reported as showing leaf symptoms similar to the yellowing in citrus, and the possibility exists that the cause is the same.

Soil Conditions.

The soils on which the above maladies occur range from very sandy to a heavy clay. In the western Transvaal, the soil is usually particularly acid—a pH of 5 to 6 being very common. In the eastern Transvaal, the soils are mostly less acid, but also definitely acid—the pH being in the vicinity of 6.

A characteristic of the soils where the above maladies occur is that in the irrigation furrows a heavy black ore deposit is always conspicuous. This same heavy black ore deposit can be easily panned out of a sample of orchard soil in much the same way as gold can be panned out of crushed gold-bearing ore.

Cause of Maladies.

"Yellow Branch."—The black deposit occurring in an irrigation furrow has been analysed for various metals. The major part of the deposit is iron in the form of magnetite, but the conspicuous result of the analysis seems to be the fact that approximately 10 per cent. of the black material is composed of chromium. This is not very surprising if it is realized that much of the citrus in the western Transvaal lies in what the geologists classify as the chromium belt, and where several chromium mines exist to-day, but there are many citrus orchards outside this belt where "yellow branch" is also very serious. Analyses of the black deposit from irrigation furrows in such orchards also showed the presence of considerable quantities of chromium, although the quantities were usually smaller than those found in the regular chromium belt.

The chromium in the soil is largely in the form of chromite and is exceedingly insoluble, so that it would appear next

to impossible for the plant to absorb much of such chromium. On analysing leaves and fruit, however, surprisingly high concentrations of chromium were found to be present. In the leaves, as much as 10 parts per million of chromium, expressed on the dry-weight basis, was determined.

Much kraalmanure is used in many citrus orchards and it is possible that additional chromium may be brought in in this way, and also that the chromium present in the soil may be made more available to the roots as a result of organic acids which may be formed during the decomposition of the manure. For this reason a few samples of kraalmanure from the western Transvaal were analysed for chromium and up to 1,303 parts per million were found to be present.

Manganese determinations were also made on the leaves in order to check up on the possibility of a manganese toxicity or deficiency. The quantities of manganese present are considered to be more or less normal, so that neither a deficiency nor toxicity is suspected. The black ore deposit in the irrigation furrows contained little or no manganese.

"Greening" or "Arrested Development."—As indicated above, the black deposit in the irrigation furrows in orchards in the eastern Transvaal, where this disease occurs, is also characteristic. The chemical analysis in this case, however, disclosed the presence of a relatively high concentration of manganese and very little chromium.

Analyses of orange leaves proved manganese to be present in exceptionally high concentrations. The chromium content, on the other hand, was much lower than in the case of leaves from trees affected with "yellow branch" in the western Transvaal.

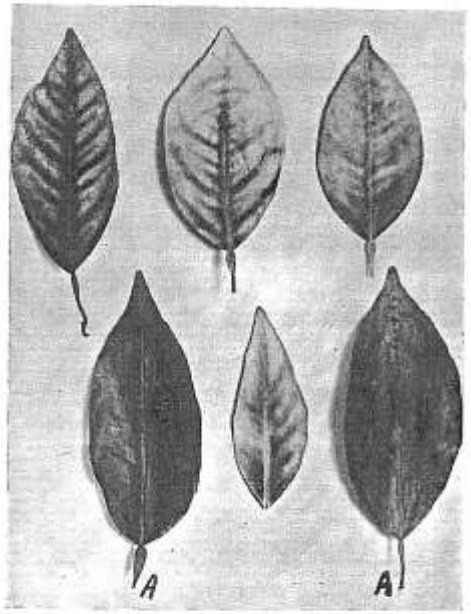


Fig. 2.—A. Normal leaves compared with mottled leaves.

Discussion.

Citrus growers are warned against jumping to conclusions from this preliminary report. Much investigational work is still in progress, and until the diseased conditions described herein can be reproduced artificially it will not be possible to conclude definitely that "yellow branch" is due to chromium toxicity, and "greening" or "arrested development" to manganese toxicity. Attempts at reproducing these diseases are in progress.

On the assumption that the above reasoning is correct, extensive investigations have also been commenced with a view to evolving methods by which chromium and manganese toxicity could be combated in the field.

Most of the chromium present in the soils of the western Transvaal is in the very insoluble chromite form. The method by which the plant absorbs chromium from this insoluble form and the question whether the plant absorbs the chromium from more soluble forms present in only small quantities, are also being investigated. The manganese present in the soils of the eastern Transvaal, however, exists in a very soluble and hence available form. Investigations are being conducted with a view to making the manganese in the soil less readily available to plants.

Acknowledgments.

The generous assistance during the early stages of this investigation of W. Kupferberger, F. C. Partridge and J. E. de Villiers of the South African Geological Survey and of T. J. W. Jurdon and C. Holzapfel of the South African Iron and Steel Corporation is gratefully acknowledged.

Peach Leaf Curl.

This disease can be most severe on some peach varieties especially when a cold wet period is followed by relatively warm and humid conditions at the time of budding and blossoming.

The colour of the infected leaves is not a normal green, but a light yellow, which later on may become red. Affected leaves become much distorted and curled or convoluted, from which the disease can be readily recognized.

The causal organism or fungus hibernates in the bud scales as well as on the trunk and branches, and infection takes place as soon as the new leaves begin to unfold after bud movement. As the leaves grow older, they become highly resistant.

All that is necessary to control leaf curl is a single application of any one of the following fungicides: Lime-sulphur in the proportion of 1 gallon to 14 gallons of water; Bordeaux mixture, 8 lb. to 50 gallons of water; or copper-sulphate, 1 lb. to 40 gallons of water.

The spraying should be done thoroughly and the fungicide applied during the winter while the trees are still dormant and before bud movement commences. It should be noted that after the trees have begun to blossom and the leaves have become infected, it is too late to spray. Also, under no circumstances should anyone of the above fungicides be applied at the prescribed concentrations to peach trees in leaf, since foliage injury will result.

(Dr. B. J. Zipperaar, Department of Plant Pathology, Stellenbosch-Elsenburg College of Agriculture.)

Studies in China (1940s)

- China- HLB became widespread in Guangdong in 1940s. Prof Lin Kongxiang surveys in southern China/research

Spread Through SE Asia

- Indonesia – 1948
- Taiwan - 1950
- Philippines - 1950s
- Thailand - 1960s
- Malaysia - 1970s

Citrus movements

- Vietnam to China -1934
- Citrus from China to Indonesia -1945
- Budwood and potted trees from China, India, Taiwan to Philippines before 1957
- Mandarin seedlings from China to Malaysia in late 1950s/60s

The vectors

- 1964 – Schwarz – suspect arthropod vector
- 1965 – McLean & Oberholzer identified vector as African citrus psyllid, *Trioza erytreae*
- 1967 – Asian citrus psyllid (*Diaphorina citri*) identified as vector in Philippines (Martinez et al.)& India (Capoor et al.)

Trioza erytreae (L) ; *Diaphorina citri* (R)

Trioza leaf damage

Pomelo psyllid
Cacopsylla (*Psylla*) *citrisuga*

adult
(lucid wings)

nymphs

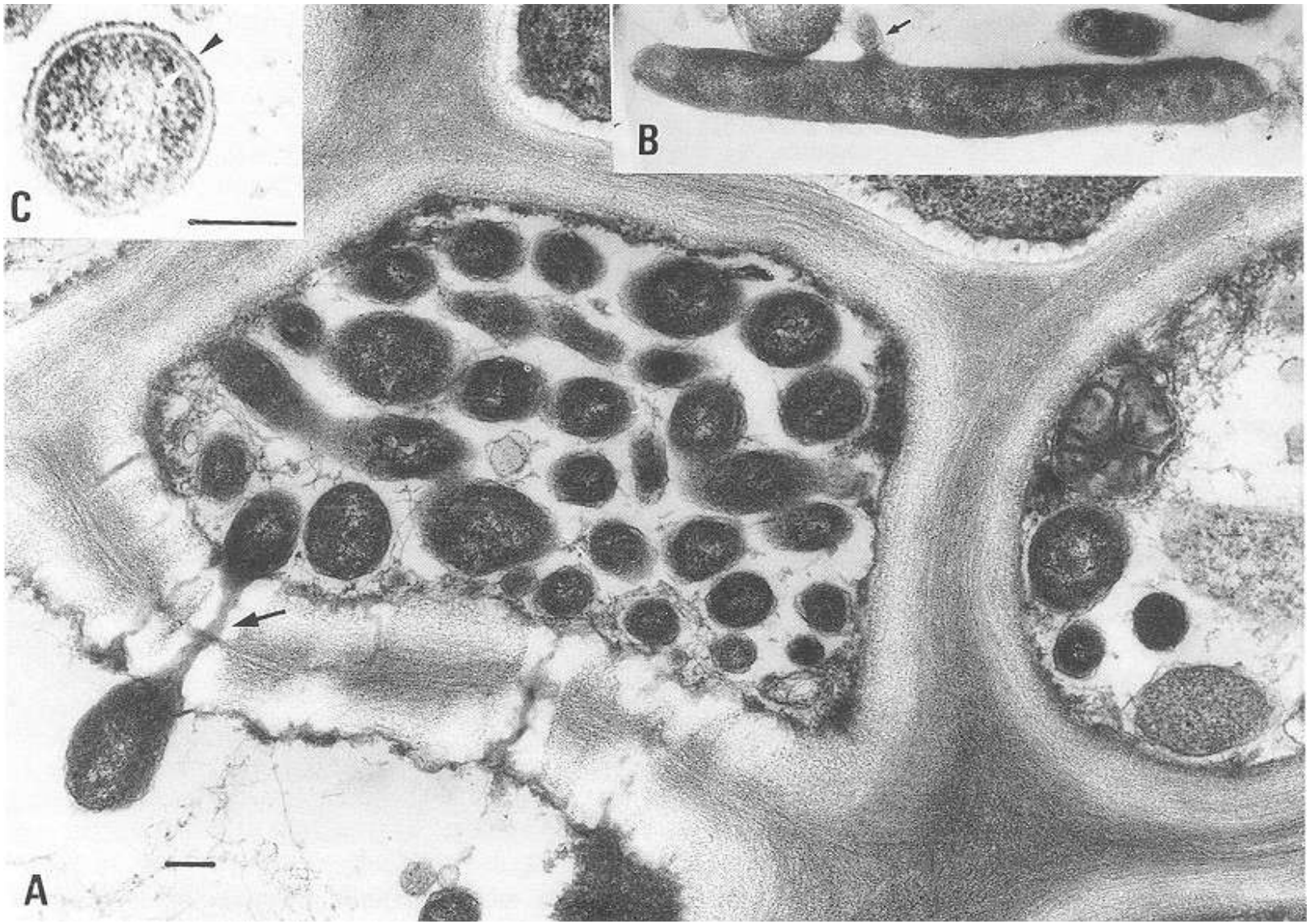
Diaphorina communis?

Trioza diospyri ?

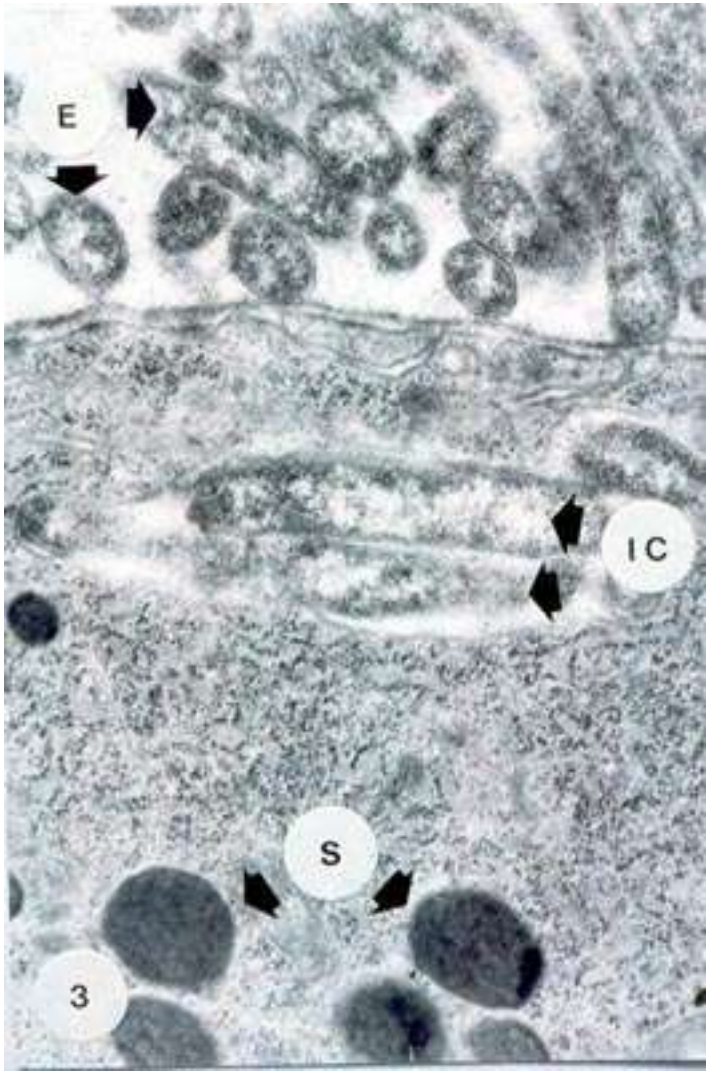
ETIOLOGY

- Root problems/ waterlogging
- Nutritional/ Toxicity
- Virus (graft & insect transmission)
- Mycoplasma-like (Phytoplasma) (initial EM of phloem)
- Bacterium

Ca. Liberibacter in phloem



Bacterial cells in psyllid (J.Moll)



Liberibacter genome (UFL/USDA)

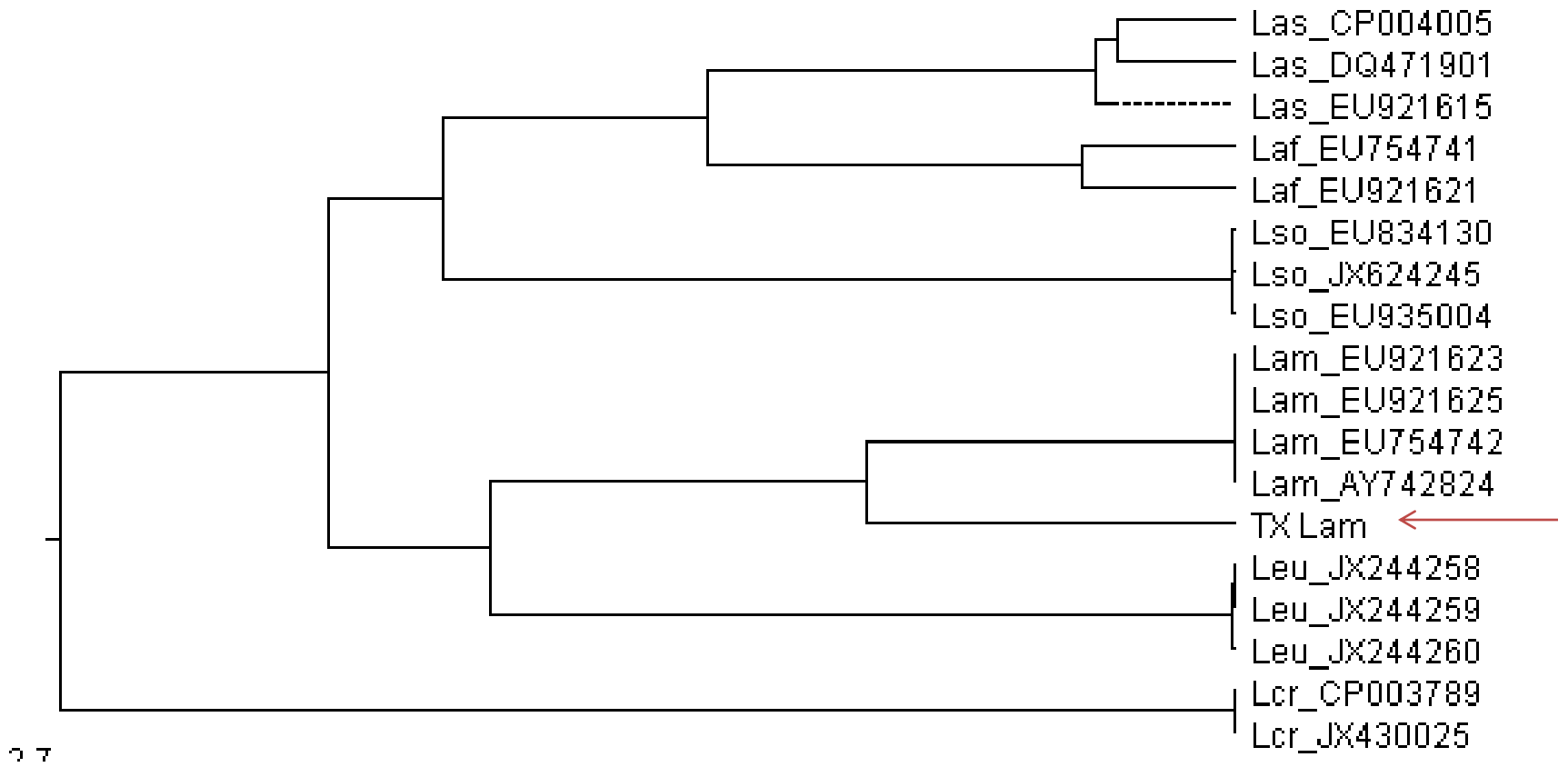
- Approx. 1.2 million base pairs
- 1,186 ORFs (836 formally assigned)
- Lacks “defensive weapons”:

  endogucanase
  galacturonase
  pectinase
  xylanase
  pectate lyase
  cellulase

Types of HLB

- 1. Asian HLB – heat tolerant; transmitted by *D. citri*. Caused by *Ca.* Liberibacter asiaticus
- 2. African HLB – heat sensitive (>30C); transmitted by *T.erytreae*. Caused by *Ca*.L. africanus
- 3.”Brazilian” HLB. Asian form + new species (*Ca.* L.americanus – heat sensitive)

Phylogenetic tree of Liberibacter spp.



Courtesy Wenbin Li, 03/27/2013

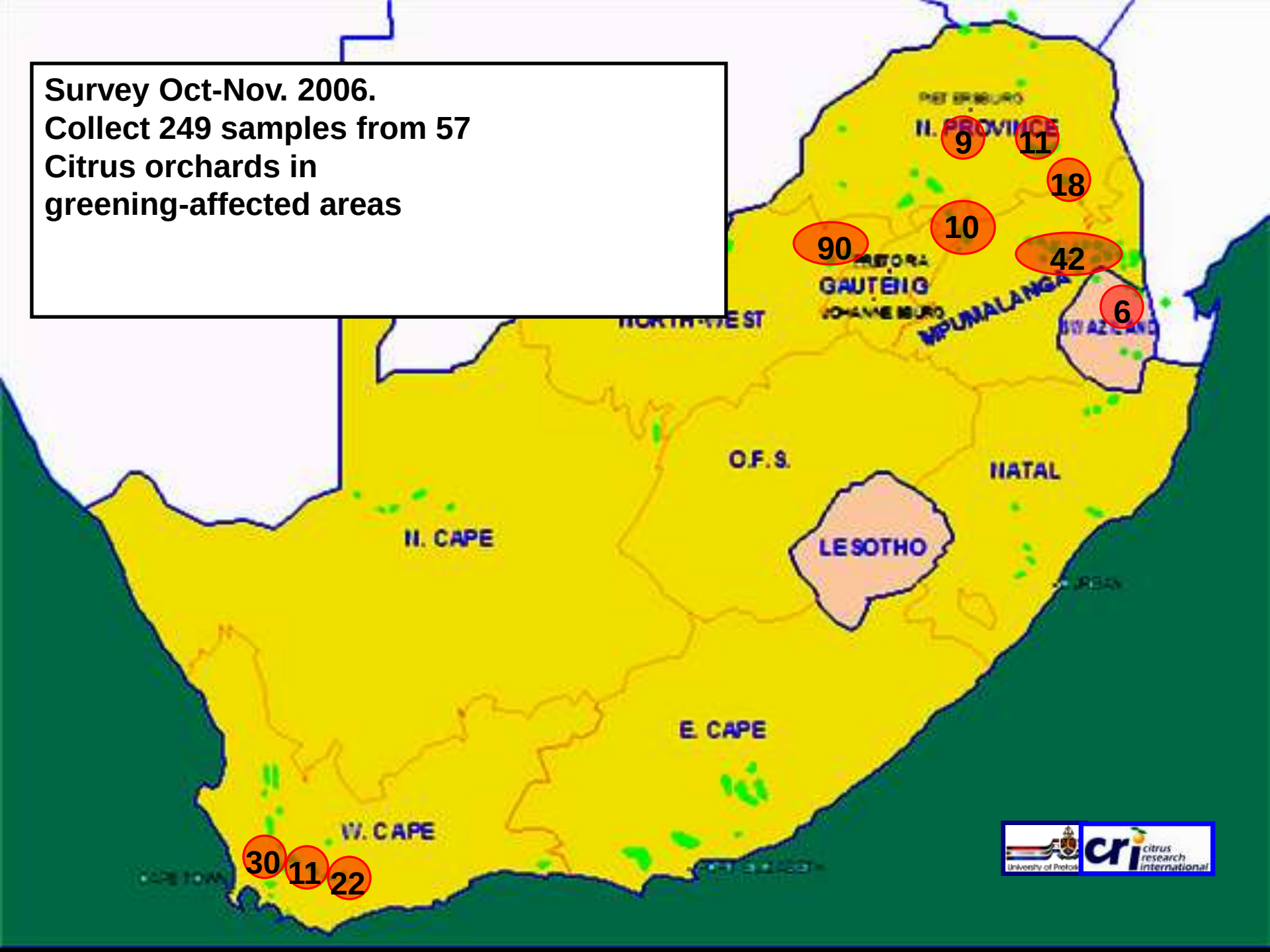
Citrus not original host

- Symptoms very severe
- Little resistance/tolerance in any species
- Citrus cultivated for 4,000 years – no HLB until recently

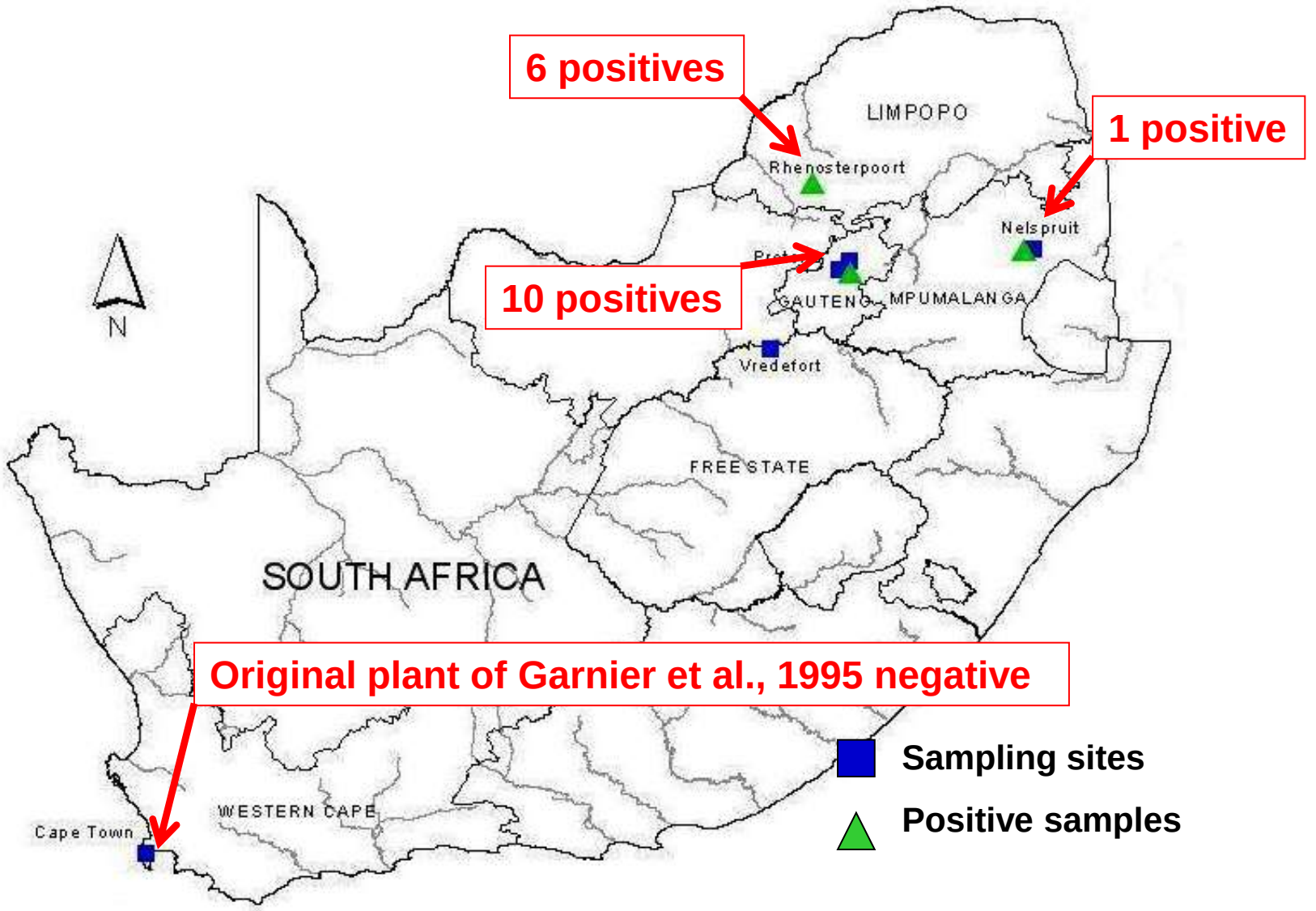
Origins?

- Laf – some indigenous Rutaceae recently identified as possible origins.
- Las – some possible candidates?
- Lam – unknown, but may be indigenous to Americas

Calodendrum capense – *Ca.* L. africanus ssp capensis


Survey Oct-Nov. 2006.
Collect 249 samples from 57
Citrus orchards in
greening-affected areas
9
11
18
10
90
42
6
GAUTENG
MPUMALANGA
O.F.S.
NATAL
N. CAPE
LESOTHO
E. CAPE
W. CAPE
30
11
22
citrus research international

Calodendrum capensis

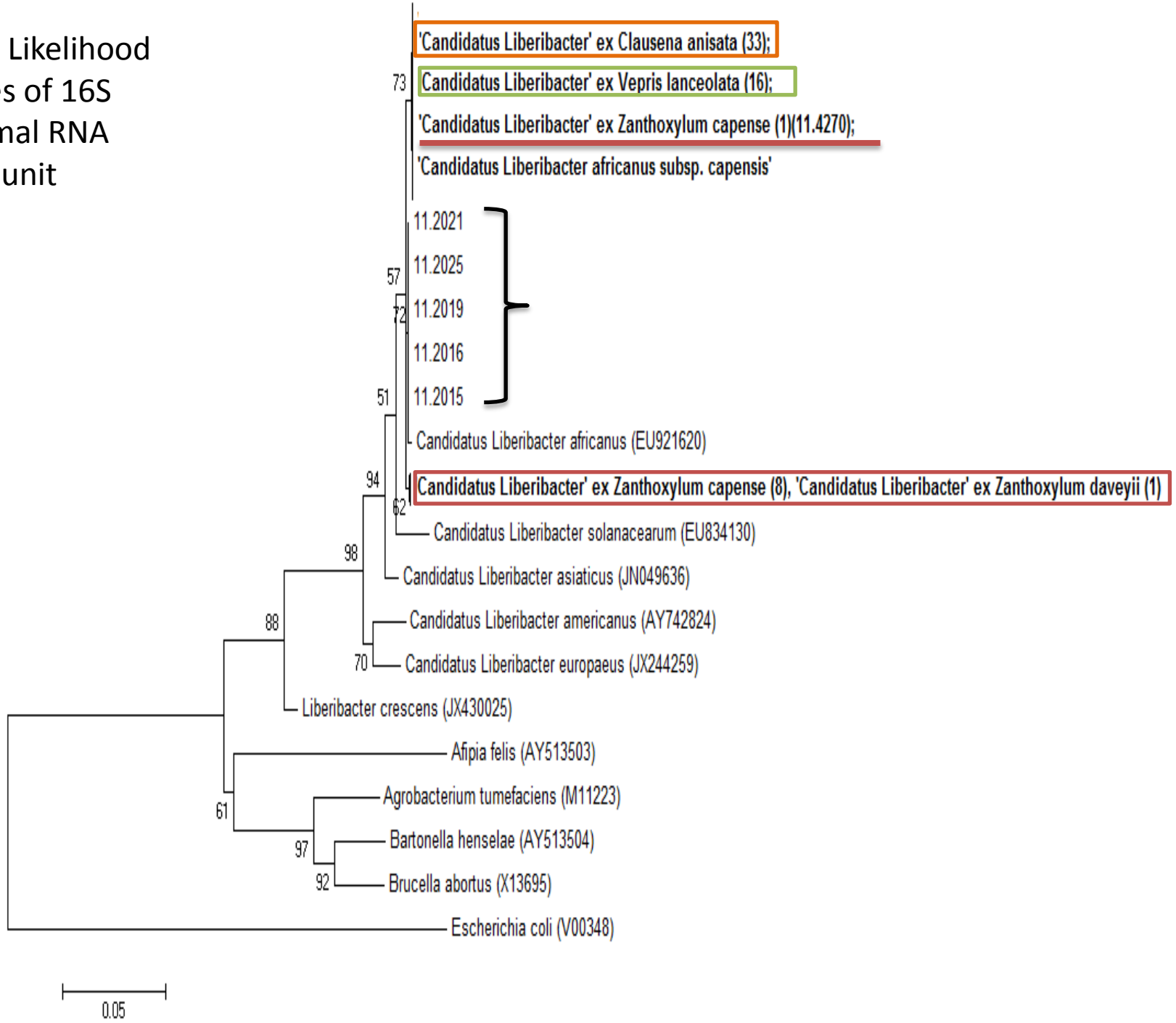


Vepris lanceolata

Clausena anisata

Zanthoxylum capense

Maximum Likelihood analyses of 16S ribosomal RNA subunit



Murraya spp., *Clausena lanceum, Severinia buxifolia* - Original hosts in Asia?

The Brazilian Lam strain has not been well established in citrus and has almost disappeared from the citrus area in Brazil 8 years after its first discovery

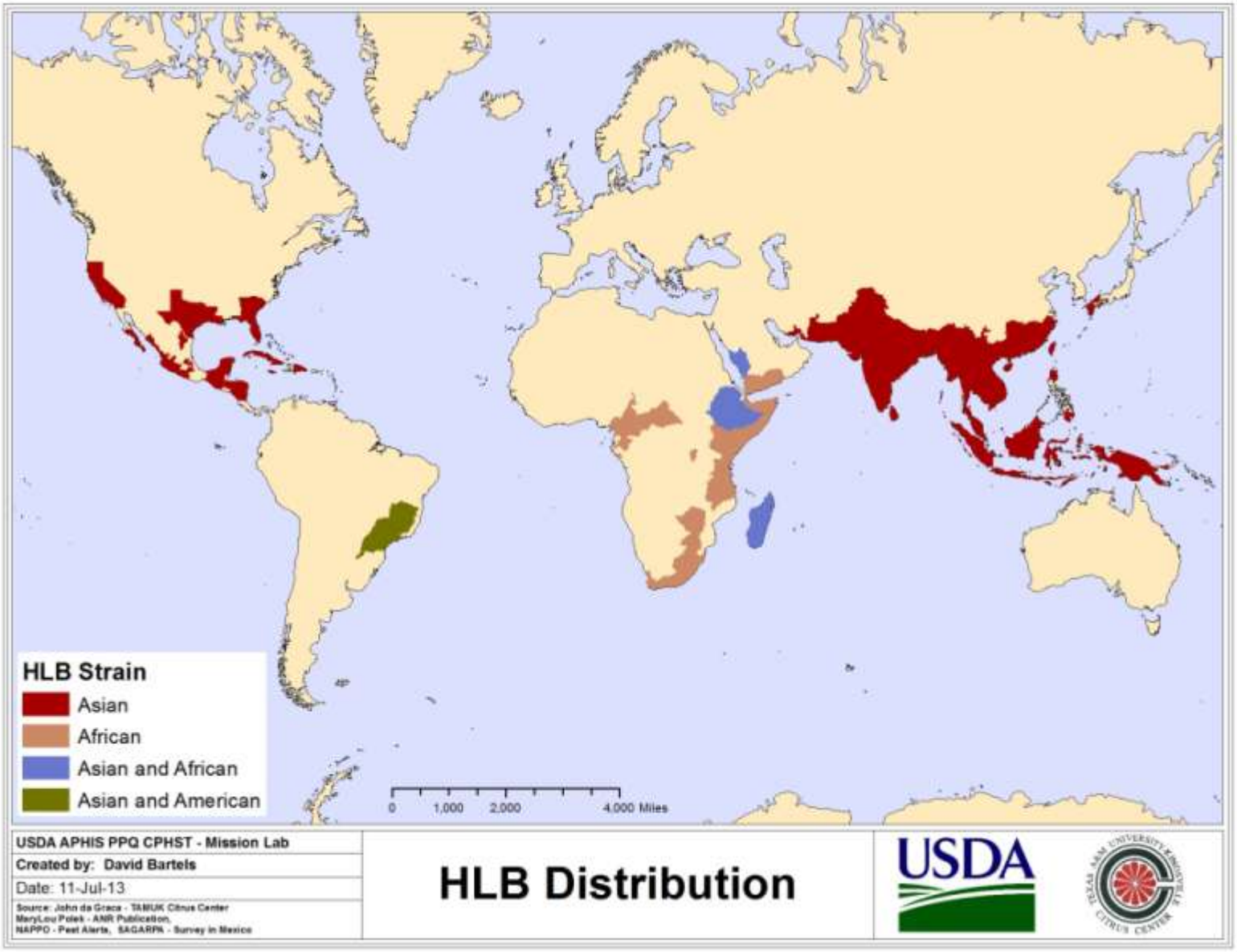
Recent movements in Asia & Africa

- Papua New Guinea – 2002; Threatens Australian citrus industry
- Iran – 2008; threatens citrus throughout Middle East and Mediterranean
- Las in Ethiopia – 2010 (1st in Africa)

Psyllid/HLB in Caribbean& N. America

Year	*D. citri*	HLB
1998	FL, Guadeloupe	
1999	Cuba, Bahamas, Venezuela	
2000	Cayman Is	
2001	TX, PR, USVI, Dom.Rep.	
2002	Campeche (MX), Belize	
2003	Tamps., NL, Costa Rica, Jamaica	
2004		
2005		Florida
2006		
2007		Cuba
2008	LA, AL, MS, GA, SC,CA	LA
2009	AZ	Jam.,Bel.,DR,Mex.-Yuc., PR, SC
2010		Mex., Pac.coast, USVI, C.Rica
2012		TX, CA

Average time from 1st Psyllid find to HLB detection = 6 years (TX – 11 yrs. CA - 4 yrs)



USDA APHIS PPQ CPHST - Mission Lab

Created by: David Bartels

Date: 11-Jul-13

Source: John da Graca - TAMUK Citrus Center
MaryLou Polek - ANR Publication,
NAPPO - Pest Alerts, SAGARPA - Survey in Mexico

HLB Distribution

USDA

TEXAS A&M UNIVERSITY KINGSVILLE CITRUS CENTER

Pigeon pea witches broom Phytoplasma –

J. M. Bove

Thank You.