BACKGROUND, CURRENT SITUATION AND MANAGEMENT OF HLB AND ITS VECTOR IN SOUTH AFRICA.

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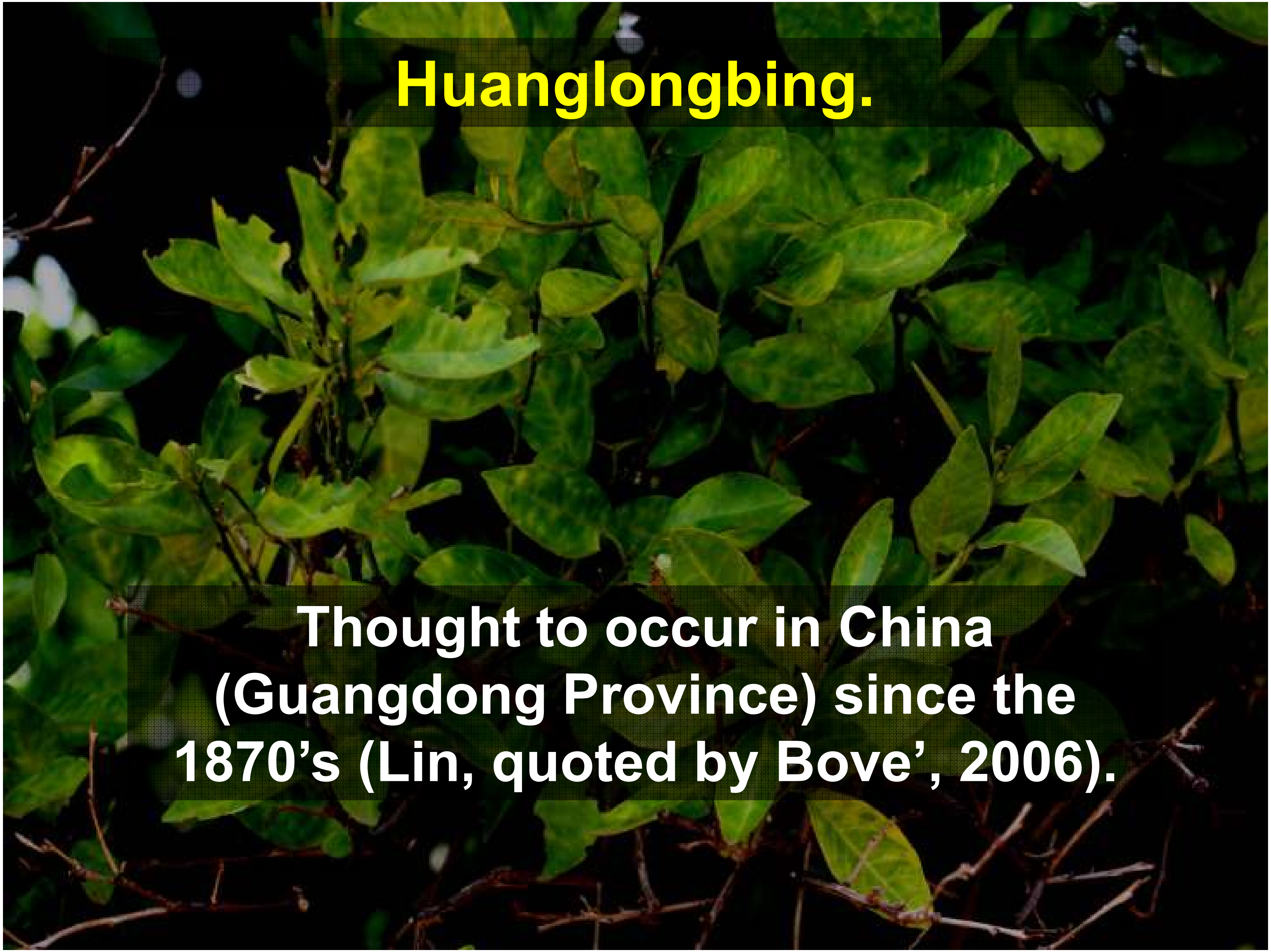
cri citrus research international


Huanglongbing.
Thought to occur in China (Guangdong Province) since the 1870’s (Lin, quoted by Bove’, 2006).

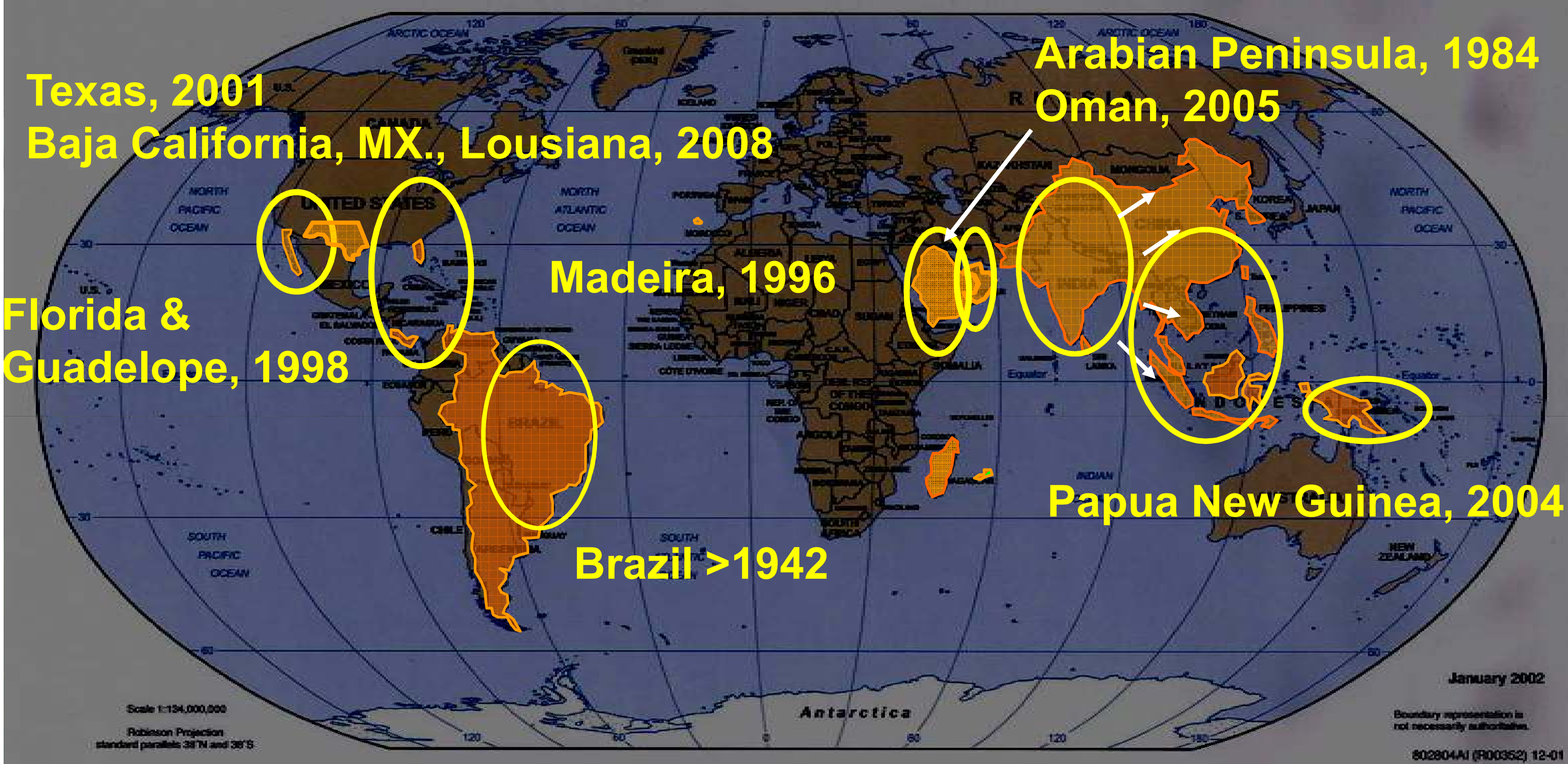
Huanglongbing.

In Asia, the disease is associated with a phloem limited, fastidious bacteria called "*Candidatus* Liberibacter asiaticus" and vectored by the pysllid, *Diaphorina citri*.

Image by GAC Beattie

Spread of *"Ca.* Liberibacter asiaticus" species to Western Hemisphere (since 2004), also mainland Africa.

World distribution of *Diaphorina citri* (vector of HLB)



Similar, but heat-sensitive disease in South Africa.

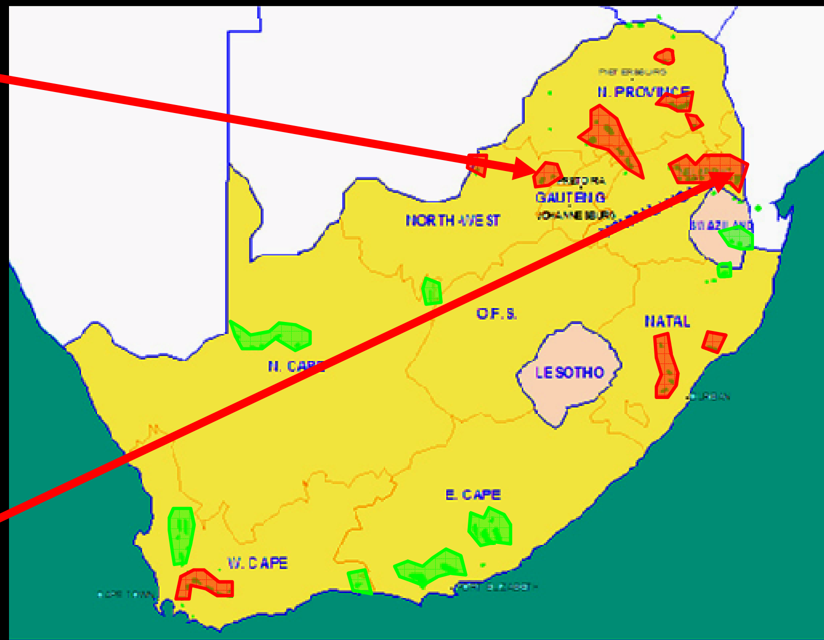
Known in South Africa since 1920's as either:

"yellow branch disease" (Brits/Rustenburg)

or

"greening"

(Eastern Transvaal).



“African citrus greening”

Symptoms:

Moderate to severe at 22 to 24°C. Disappear above 27°C in laboratory trials.

“Asian citrus greening”

Symptoms expressed over both temperature regimes.

(Bove’ et al., 1974) .


“Candidatus *Liberibacter asiaticus”*
***NOT** found in South Africa.*
“Candidatus *Liberibacter africanus”*
associated with greening here.

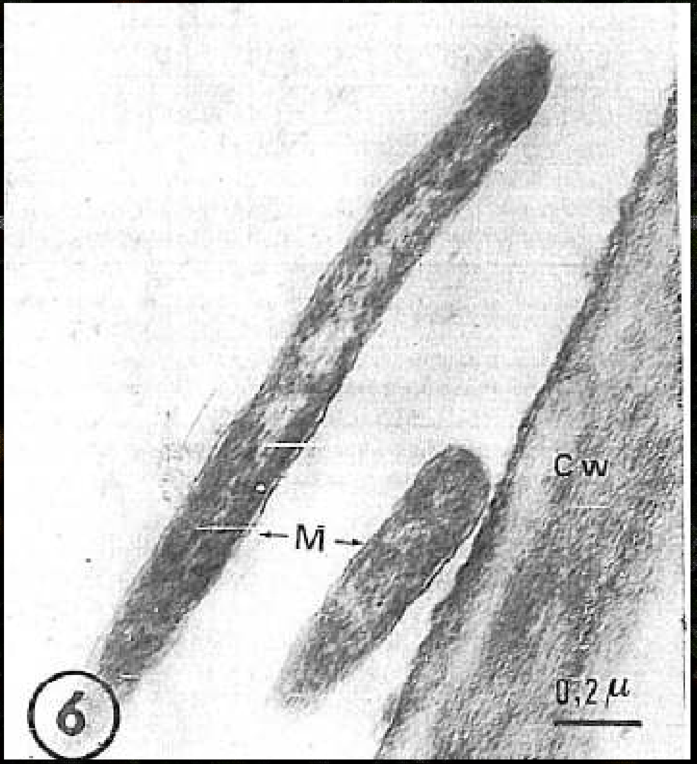
_Ca._ L. africanus and _Ca._ L. asiaticus both in the alpha- subdivision of the protobacteria.

Gram negative

Obligate intercellular parasites

Fastidious bacteria

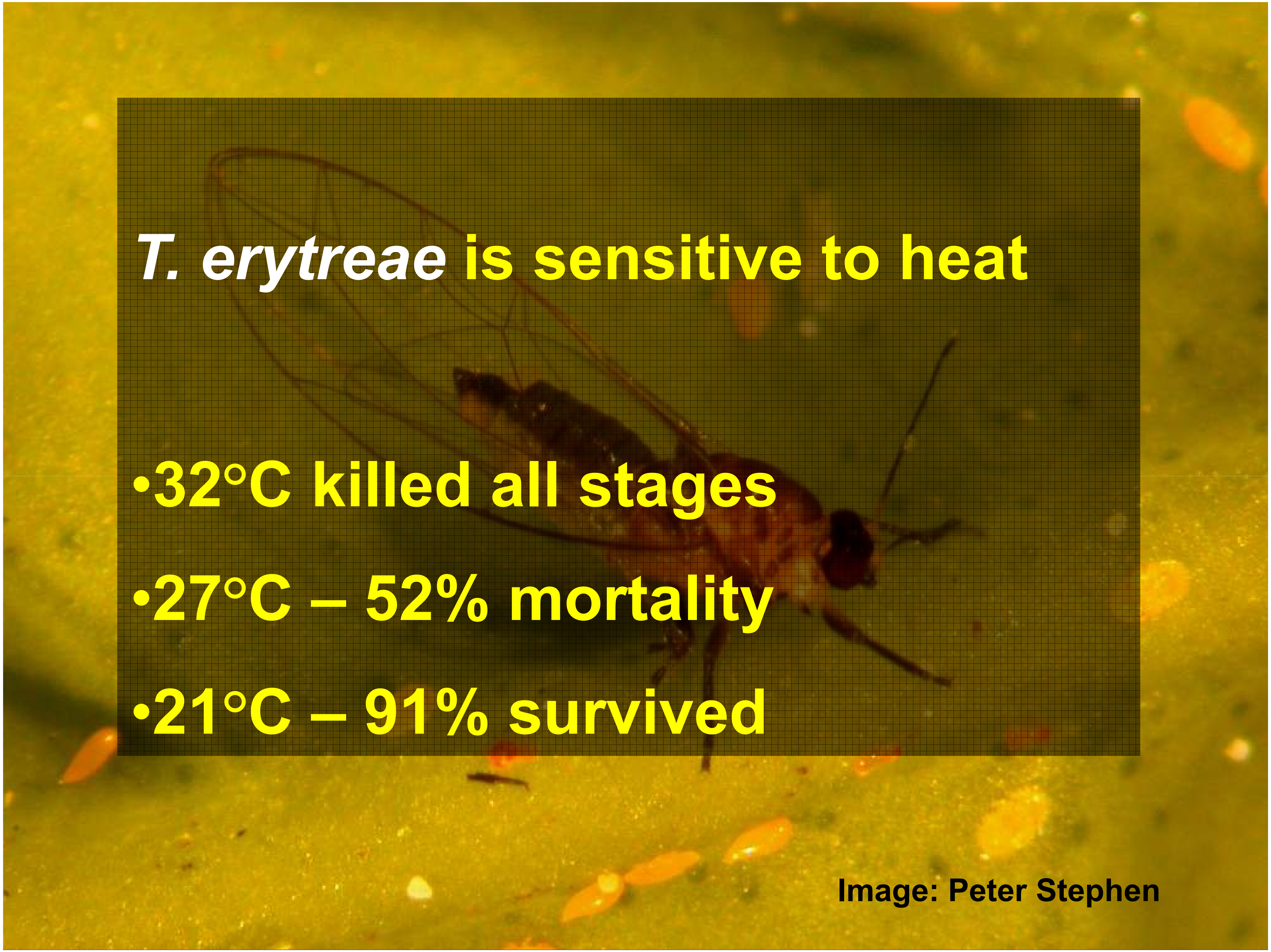
Phloem restricted



MLO (=Ca. L. africanus) in phloem.
(Image: Moll & Martin, 1973)

Diaphorina citri does **NOT** occur in South Africa and greening is spread naturally by:

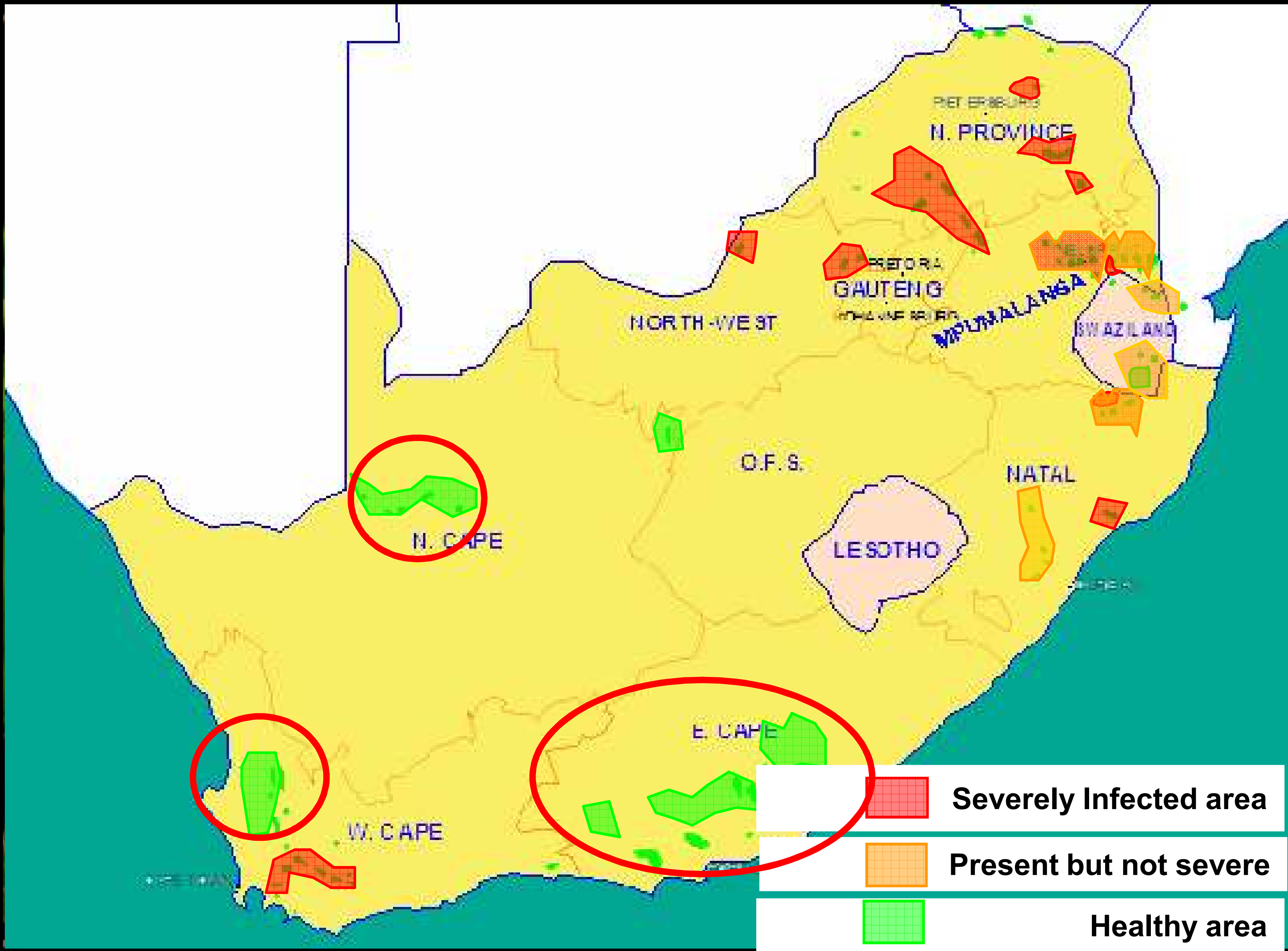
Trioza erytreae (del Guerco)


T. erytreae is sensitive to heat
•32°C killed all stages
•27°C – 52% mortality
•21°C – 91% survived
Image: Peter Stephen

Greening in South Africa.

Disease primarily found under cool conditions (25°C). Symptoms more severe at altitudes above 700m, absent or mild in low lying hot areas.

Consequently not a problem in hotter, drier areas.
Distribution, 2006




Sector of tree, derived from
a single branch, showing
yellowing and leaf symptoms.

Yellowing and blotchy mottling of leaves.

Zn- and Fe-deficiency -like symptoms, yellow midribs & small, upright leaves.

Photo's: F. van Vuuren

Lopsided fruit.

Aborted seedlings.

Colour inversion with ripening.

Silver caste when pressed.

Bitter tasting fruit.

Lower Photo's: F. van Vuuren & Peter Stevens


Peter Stephen
CRI

Economic Importance: (South Africa)

Severe incidence from 1932-1936,

1939-1946,

1958-late 1960’s,

Three major citrus production areas abandoned due to greening

Decline in incidence from the late 1960’s

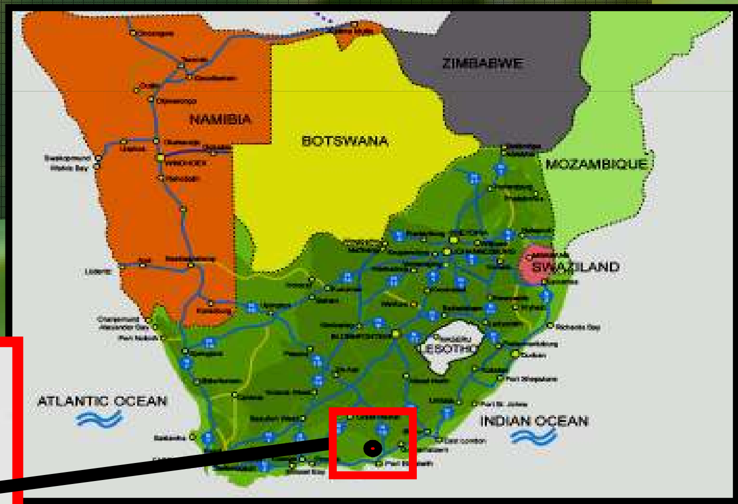
Return to production in abandoned areas.

In recent times record incidences up to 25% in orchards in some previously abandoned areas

Control through: 1) Citrus Certification Scheme: Greening free budwood material

Shoot tip grafting (ITSC & CRI, Nelspruit)

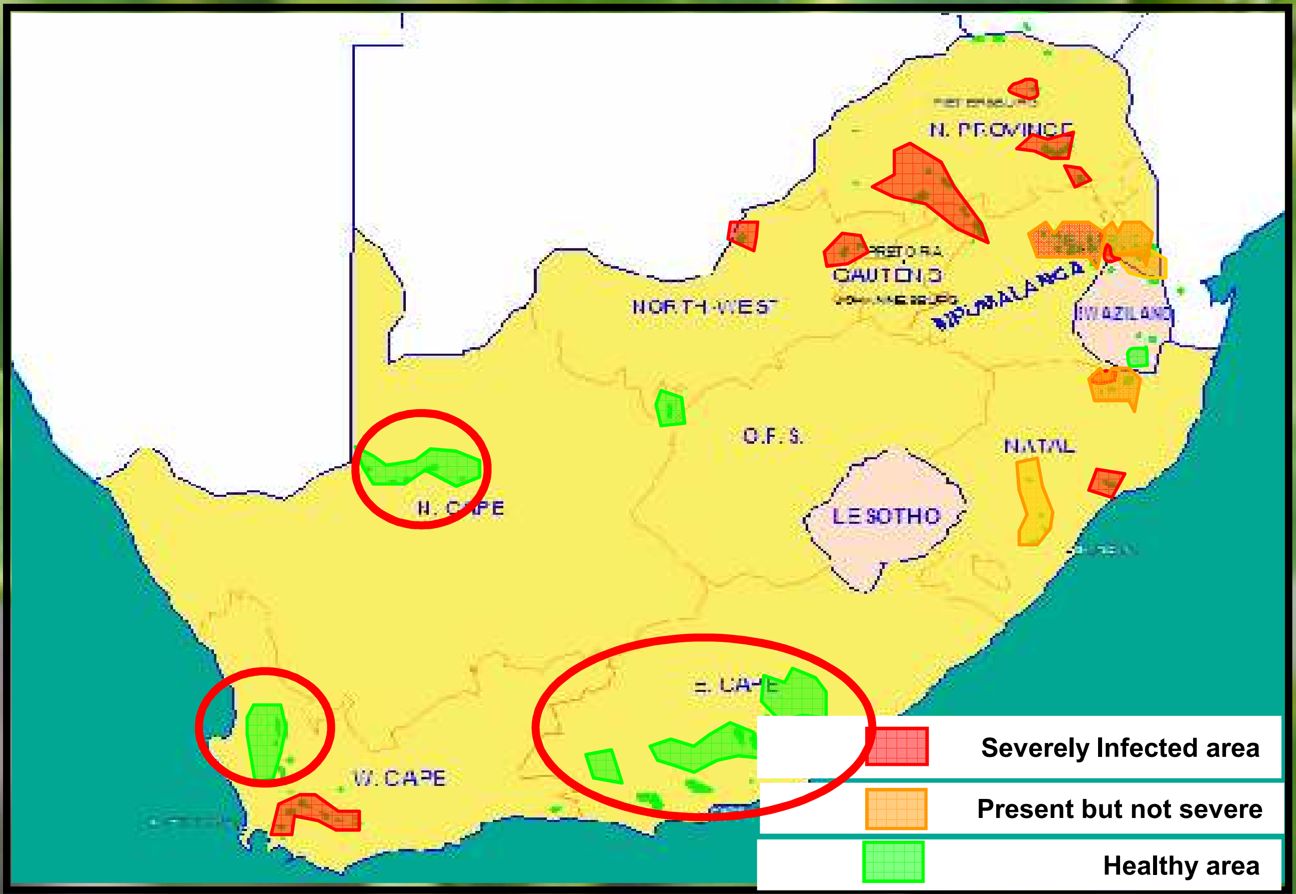
- Budwood multiplication (Citrus Foundation Block, Uitenhage)
  - Greening disease free area
  - Vector limited
  - Psylla screen netting



Citrus Foundation Block, Uitenhage

University of Pretoria

Control through: 2) Restriction on planting material from greening affected areas to greening-free regions in South Africa.



Control through: 3) Inoculum removal

Slow spread and/or Low incidence of greening in an area:

Remove infected trees *in toto*

Area in which disease is endemic and spread rapid:

Cut back branches/infected sectors

Cut back infected branch BUT control re-growth.

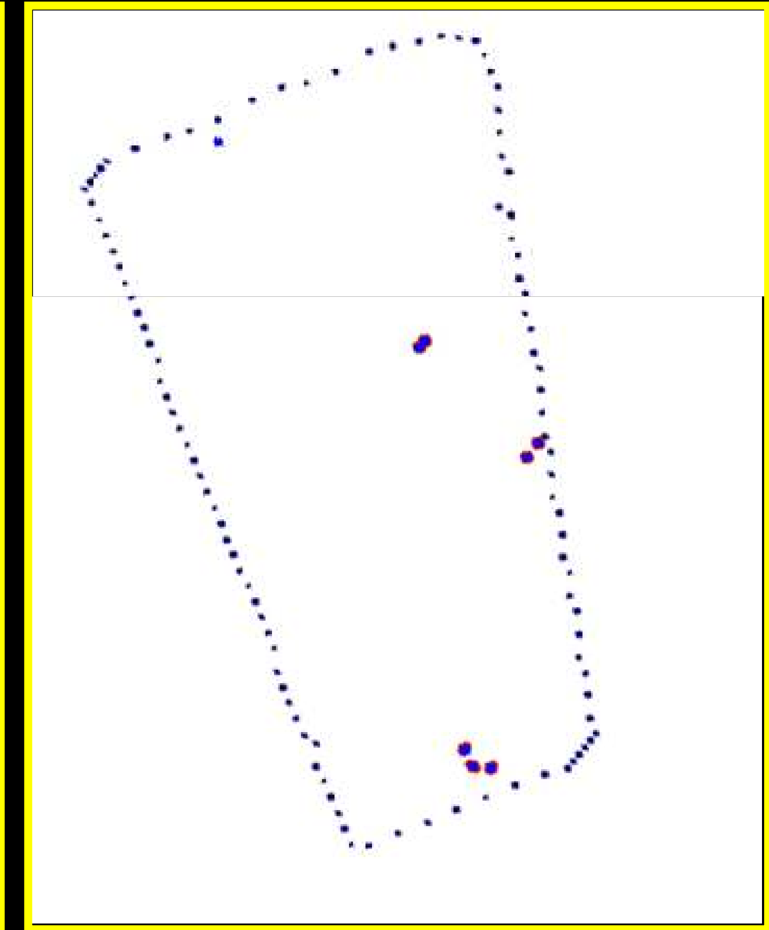
Control through: 4) Control of vector

<u>**Systemic trunk applications**</u>

- Monocrotophos (Monostem® ;Azedrin®)
  - 28 days protection (now banned)
- Methamidophos (Citrimet®)
  - 21 days protection
- Imidacloprid (Confidor®); chloro-nicotynyl
  - 56 days protection (stem)
  - Soil application c. 110 days protection
- Acetamiprid (Mospilan®); acetamidine
  - 42 days protection


Relatively effective control, sometimes difficult to find find psylla for experiment.
Image: Peter Stephen

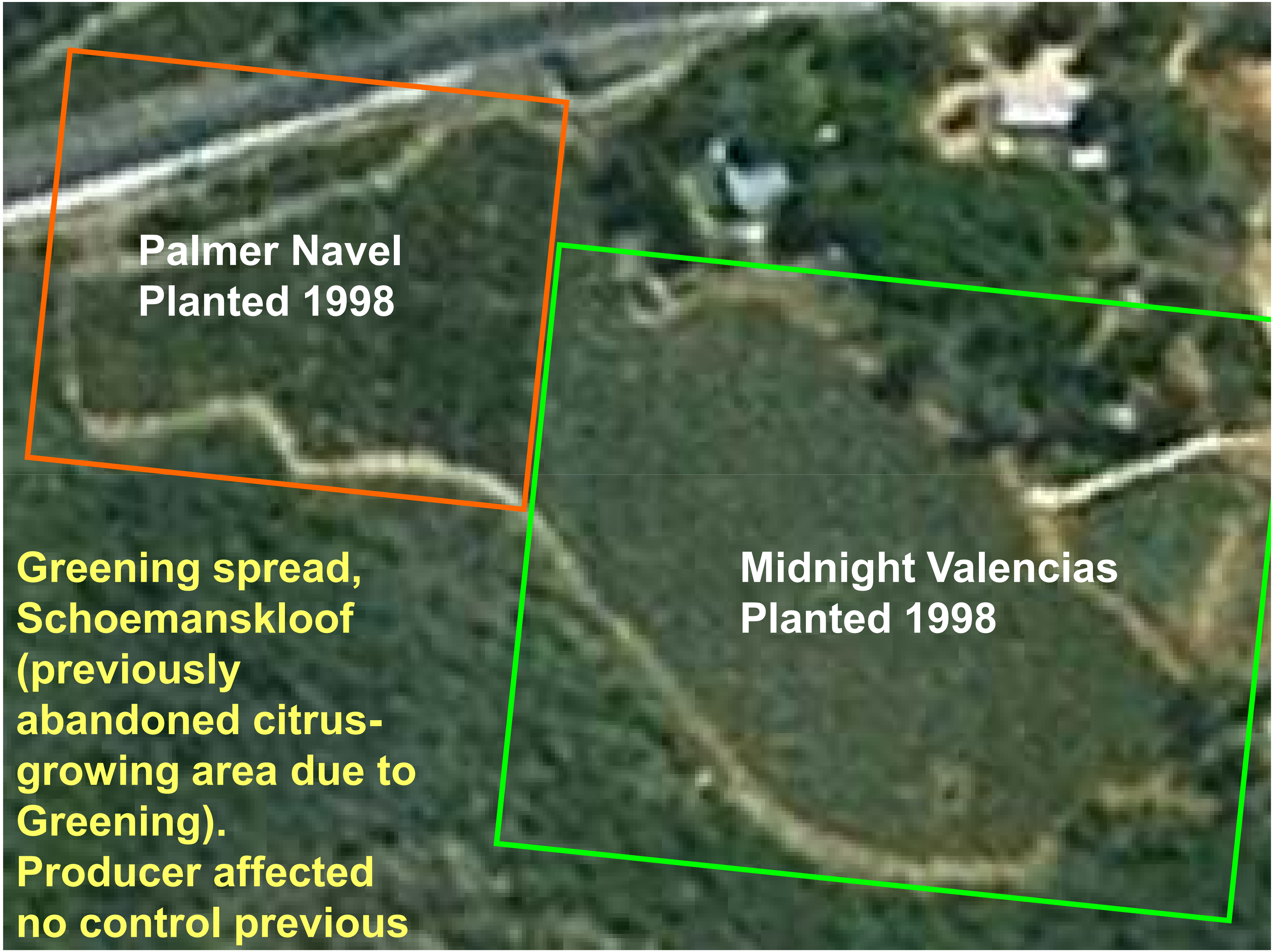
Greening spread, Wolhuterskop, Brits/Rustenburg: 2008 (7 trees), 2009 (8 trees). Producer chemically controls *Trioza erytreae*



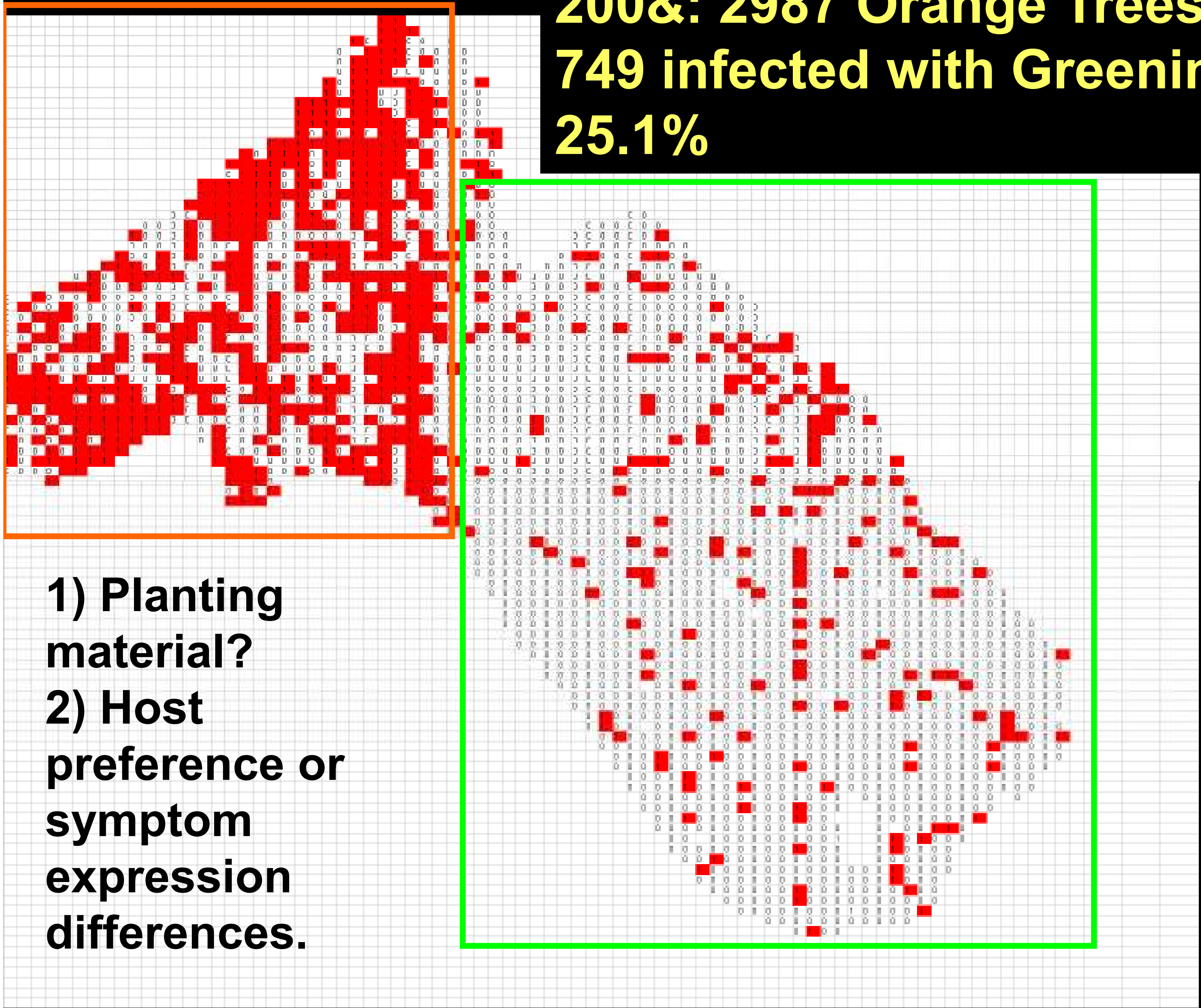
Greening spread, Nelspruit:

Various Navel cultivars.
Total of 692 Trees.
Planted 2004.
2007 (1 infected tree)
2008 (1 infected tree)
2009 (1 infected tree)

Producer chemically controls *Trioza erytreae*

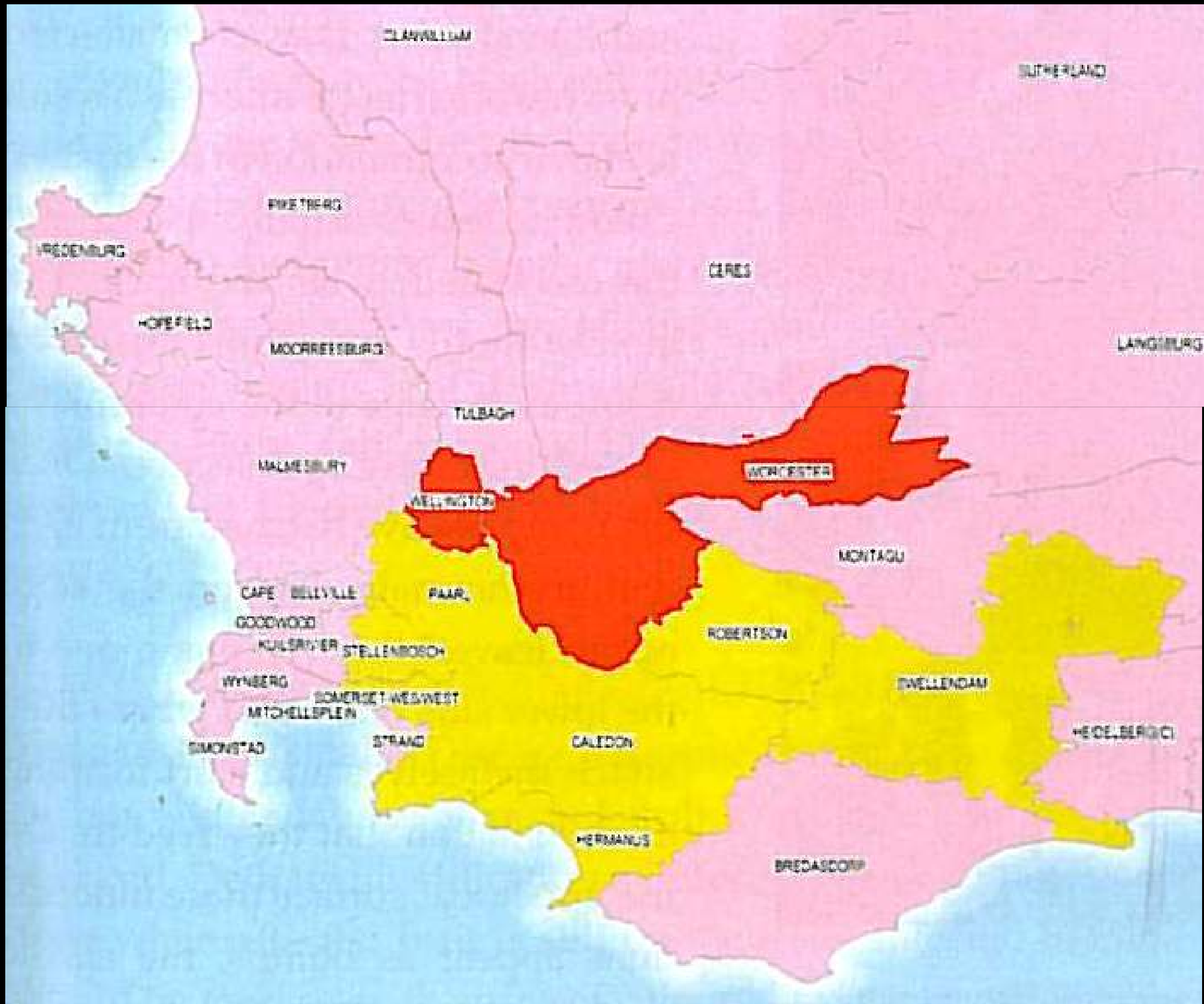

Palmer Navel
Planted 1998
Midnight Valencias
Planted 1998
Greening spread, Schoemanskloof (previously abandoned citrus-growing area due to Greening). Producer affected no control previous

200&: 2987 Orange Trees. 749 infected with Greening 25.1%



1) Planting material?
2) Host preference or symptom expression differences.

Expansion of greening affected areas in the Western Cape, 2006



Research at CRI directed at Greening.

Fanie van Vuuren: Greening infected fruit infected but displaying healthy sectors (Chimeras).

Image: J. Meyer

Fanie van Vuuren.

Recover embryos from desired sections of the chimera and culture *in vitro* to generate plants – establish clones and multiply budwood

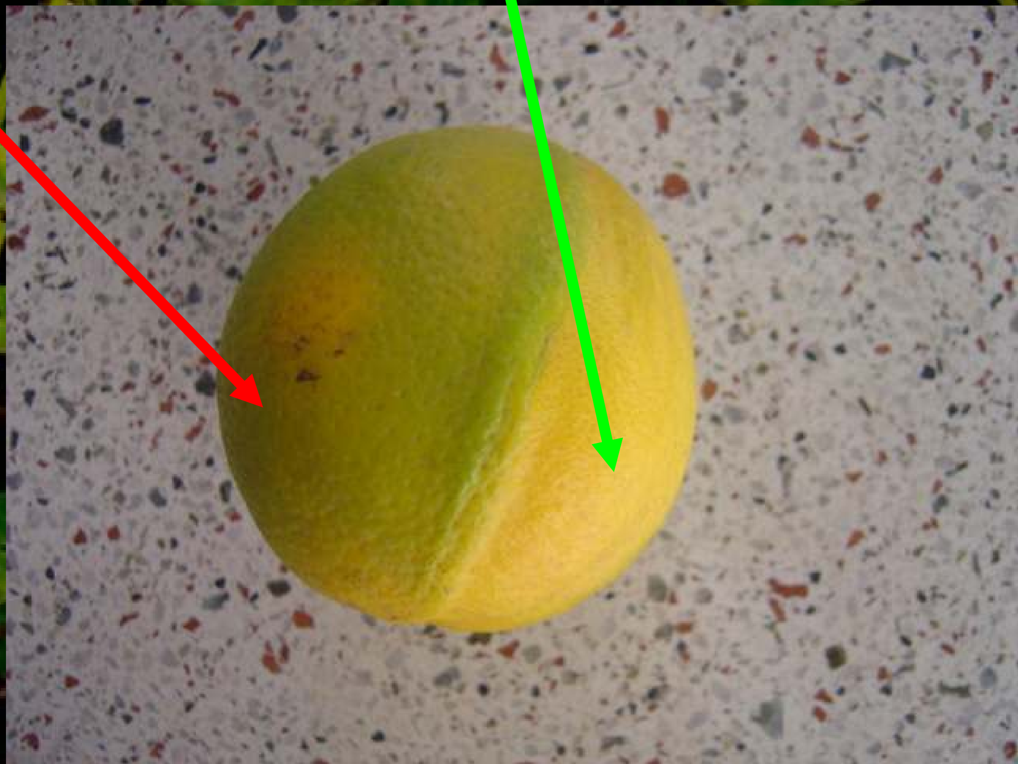
Image: J. Meyer

Compare genomes on molecular level-

Identify differences (Mutation).

Use genes in transgenic resistance approach?

Infected versus Non-infected



Foreground Image: J. Meyer

Spread in 2000's to new countries by *Candidatus* Liberibacter asiaticus.

Survey to determine if Liberibacter species, other than Liberibacter africanus are in South Africa.

Disease since late 1920's.

No surveys done in South Africa since advent of "Ca. Liberibacter" species-specific PCR techniques.

Similarity of symptoms in cool areas.

HLB ("Las") Poona transmissible by both Diaphorina & Trioza experimentally

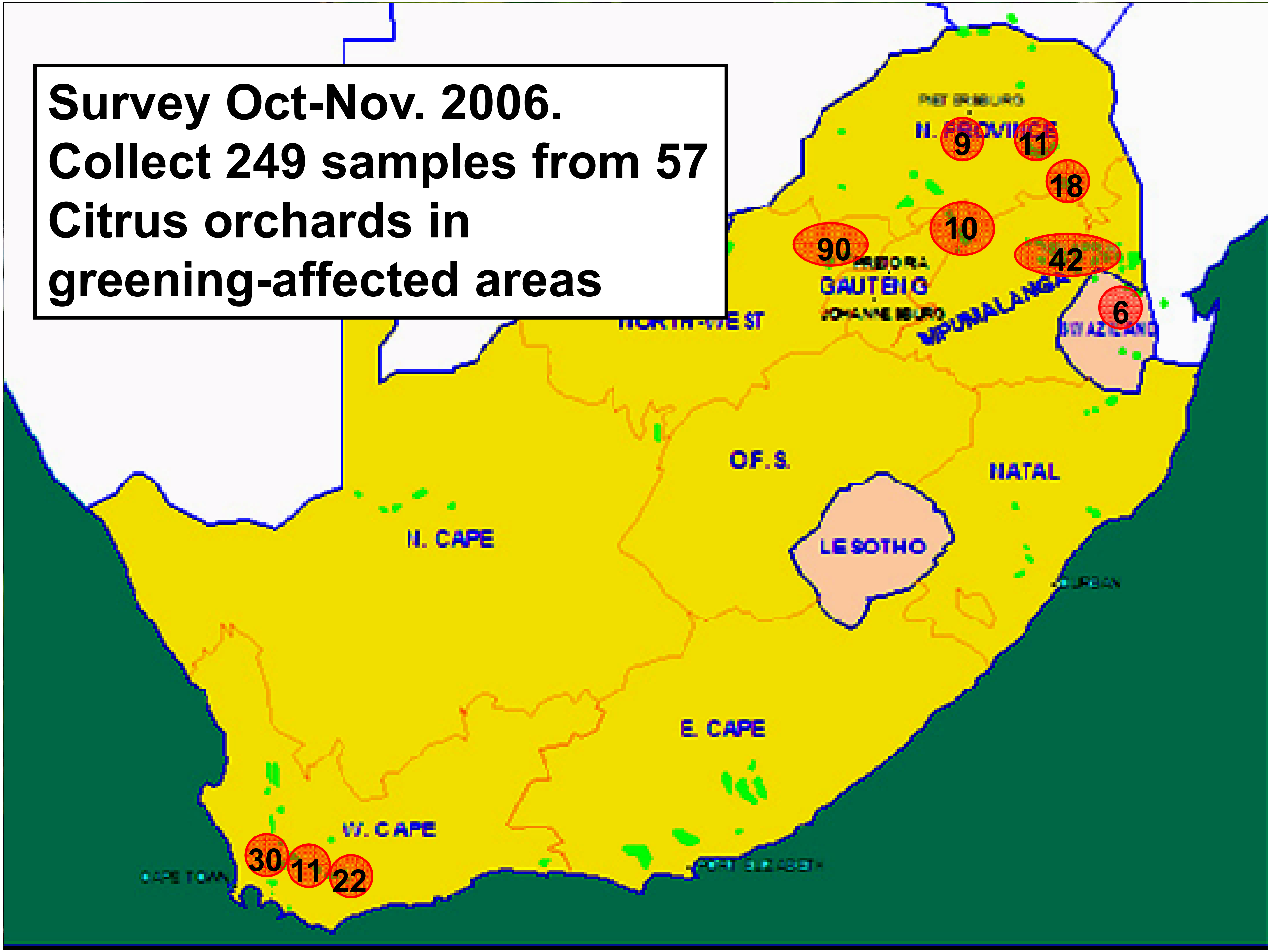
Massonie et al., 1976

Presence of indigenous *Diaphorina spp*. in South Africa

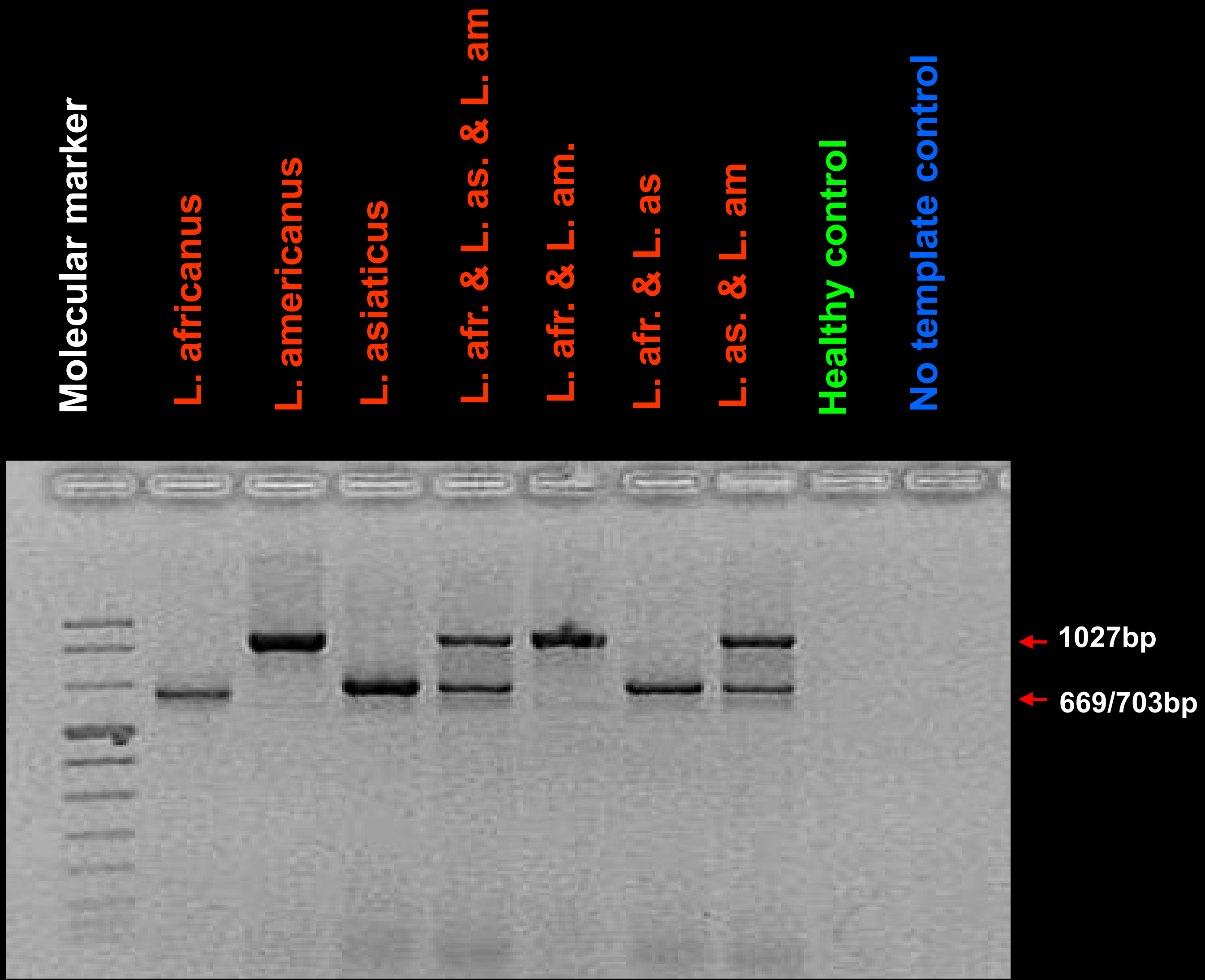
- ***Diaphorina punctulata***
  - **From *Sclerocarya caffra* (Sond.), *Clausenia inequalis* (=*anisata*), *Chorda caffra* (Boraginaceae). (Petty, 1924)**
  - **On citrus in Swaziland, greening non-vector (Catling & Atkinson, 1974).**

- ***Diaphorina zebrana***
  - **From *Ozaroa paniculosa, O. reticulata*.**
  - **On citrus in Swaziland, greening non-vector (Catling & Atkinson, 1974)**

Survey Oct-Nov. 2006.
Collect 249 samples from 57 Citrus orchards in greening-affected areas

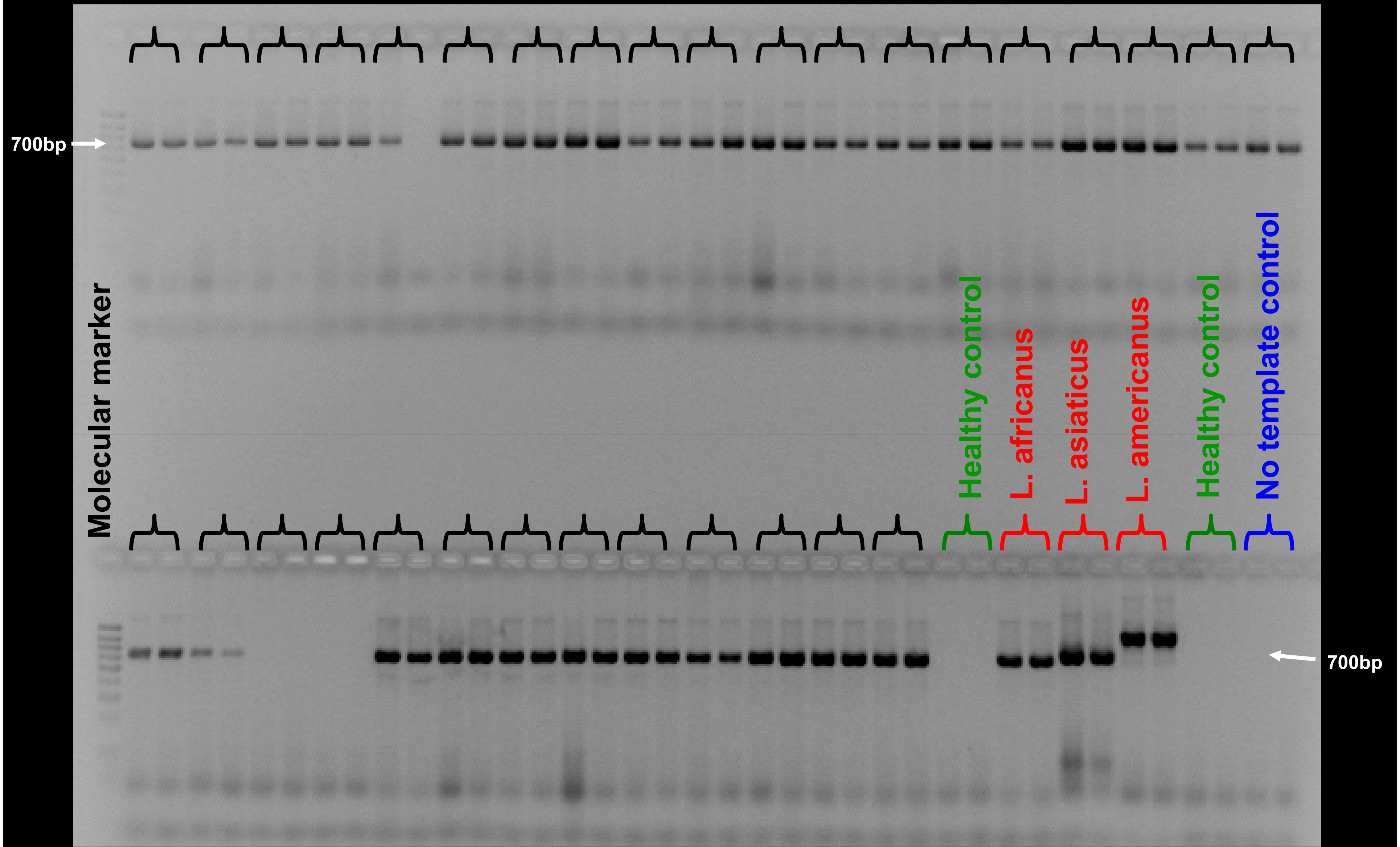


Multiplex PCR consisting of primers A2/J5[1] and GB1/GB3[2]

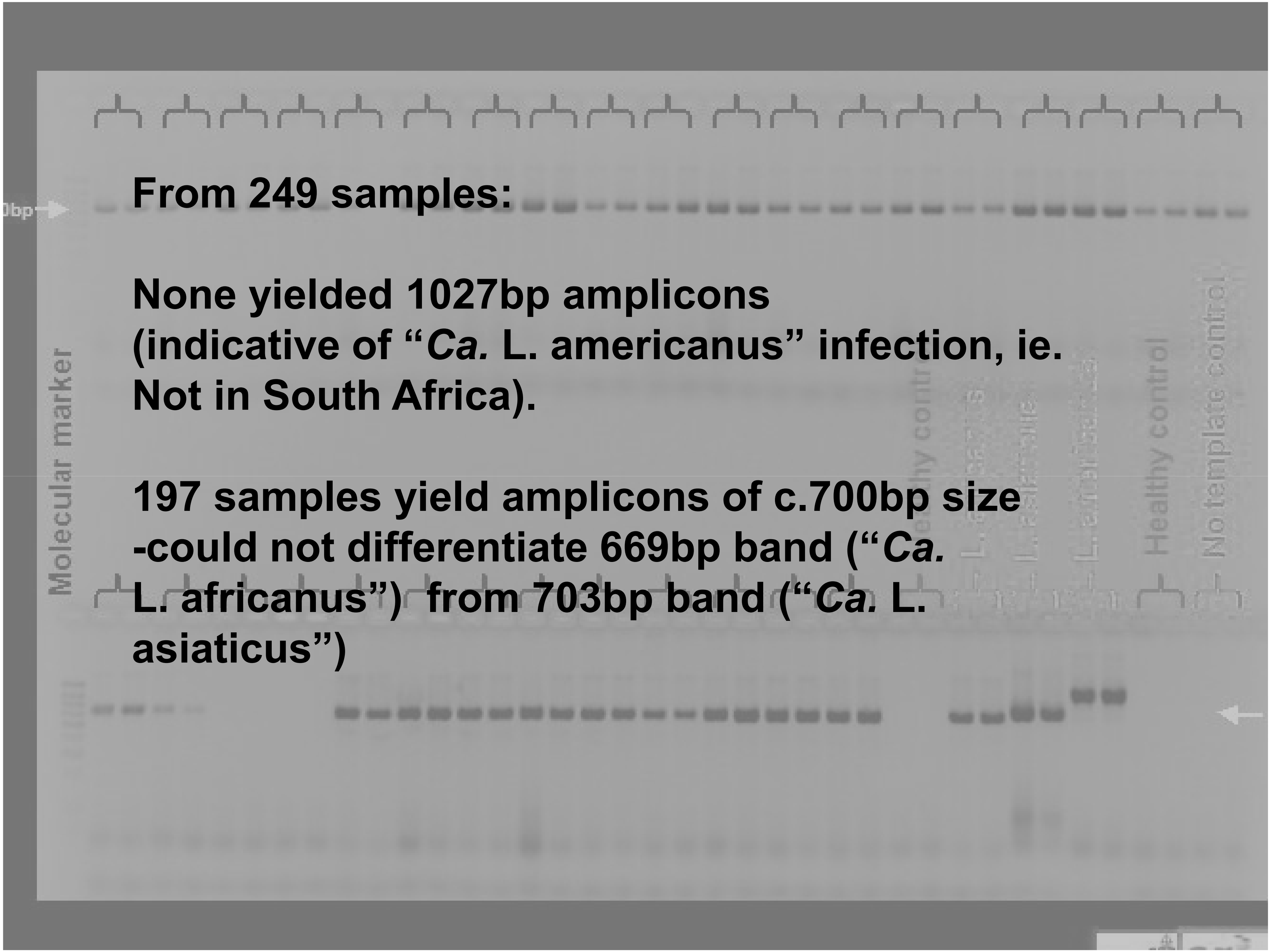


[1]Hocquellet et al., 1999; [2]Teixeira et al., 2005

Typical gel of multiplex PCR (primers A2/J5[1] and GB1/GB3[2])



[1]Hocquellet et al., 1999; [2]Teixeira et al., 2005



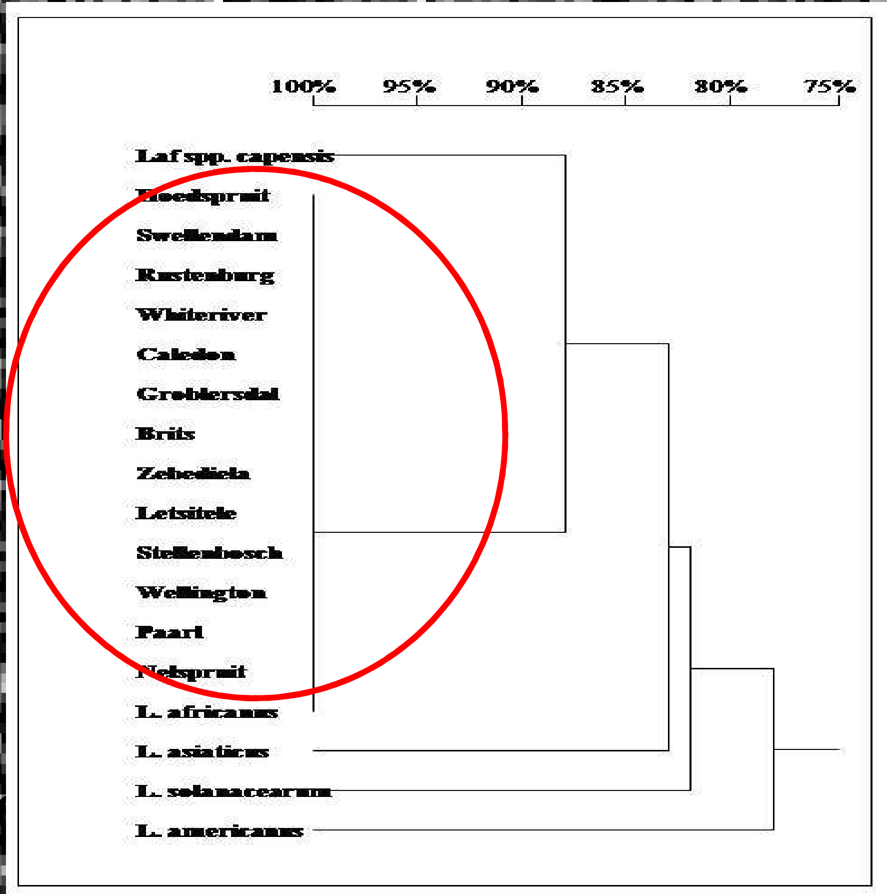
From 249 samples:

None yielded 1027bp amplicons (indicative of "*Ca.* L. americanus" infection, ie. Not in South Africa).

197 samples yield amplicons of c.700bp size -could not differentiate 669bp band ("*Ca.* L. africanus") from 703bp band ("*Ca.* L. asiaticus")

Do direct sequencing (no cloning) of A2/J5 amplicon of 84 sources.

Single forward primer and single reverse primer sequence.



All sequences "*Ca*. L. africanus" (LAU09675)

No variation.

No "*Ca.* Liberibacter africanus spp. capensis"

"*Candidatus* Liberibacter africanus spp. capensis"

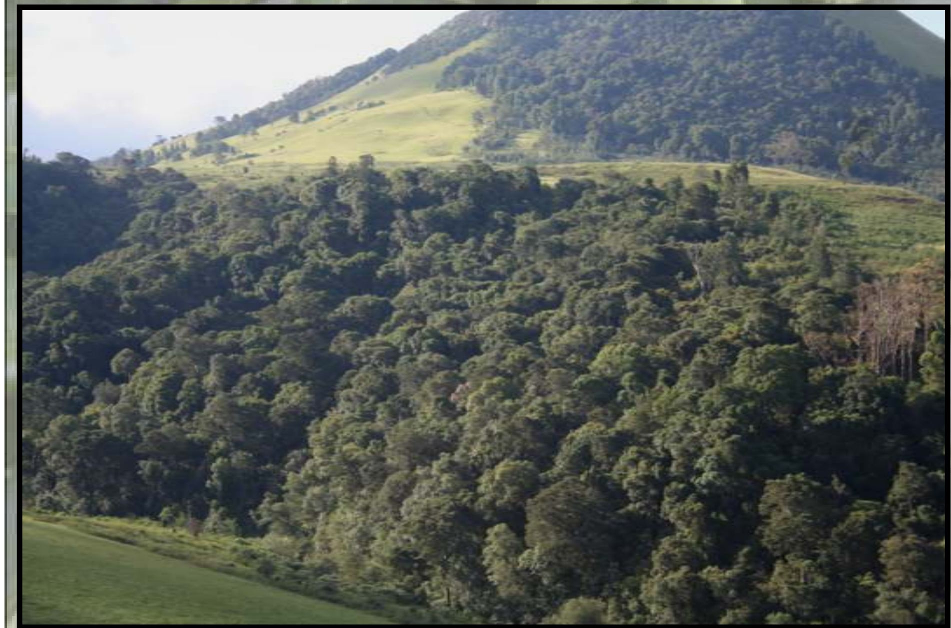
On Calodendrum capense (Cape chestnut)

detected in 1995 (Garnier *et al*., 1999)

Calodendrum capense (Rutaceae)

It occurs along the south and east coast of southern Africa from around Swellendam in the Western Cape through the Eastern Cape, KwaZulu-Natal, Mpumalanga, Swaziland, Gauteng, North West and Northern Province and into tropical Africa as far north as Tanzania and Ethiopia.

It grows mainly in forests and ravines / gorges, but occasionally in scrub and riverine bush, from sea-level to 2000 m. When in bloom, the whole canopy turns pink and is relatively easy to see in indigenous forests .



In a forest environment, *Calodendrum capense* can reach heights of up to 20m.

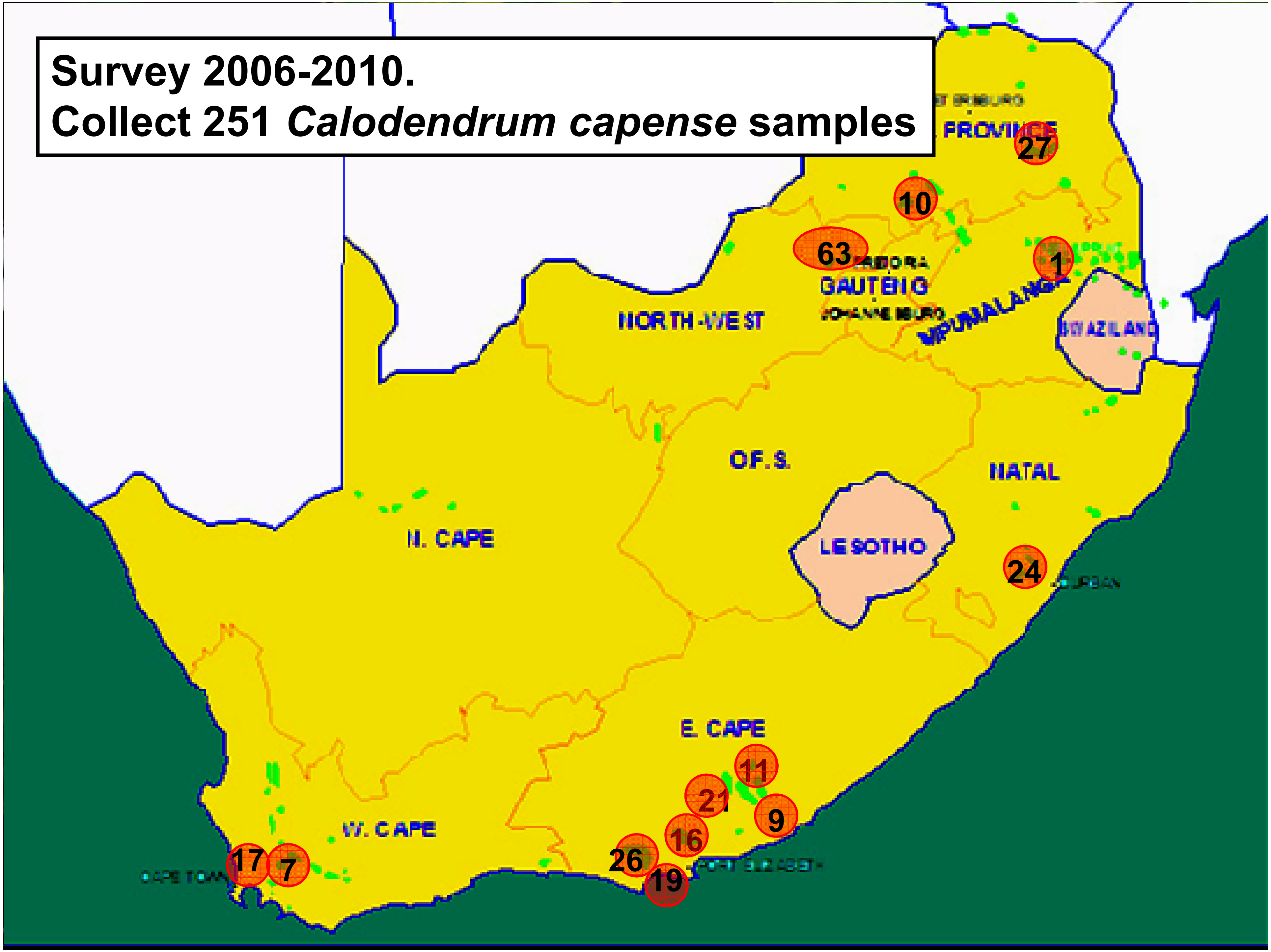
At the forest margin, in the open, and in gardens it is shorter, approx. 7 m, with a more spreading canopy. In general this is a handsome well-shaped tree with a single trunk and a dense rounded canopy.

Survey for Liberibacter spp. in indigenous Rutaceous plants:

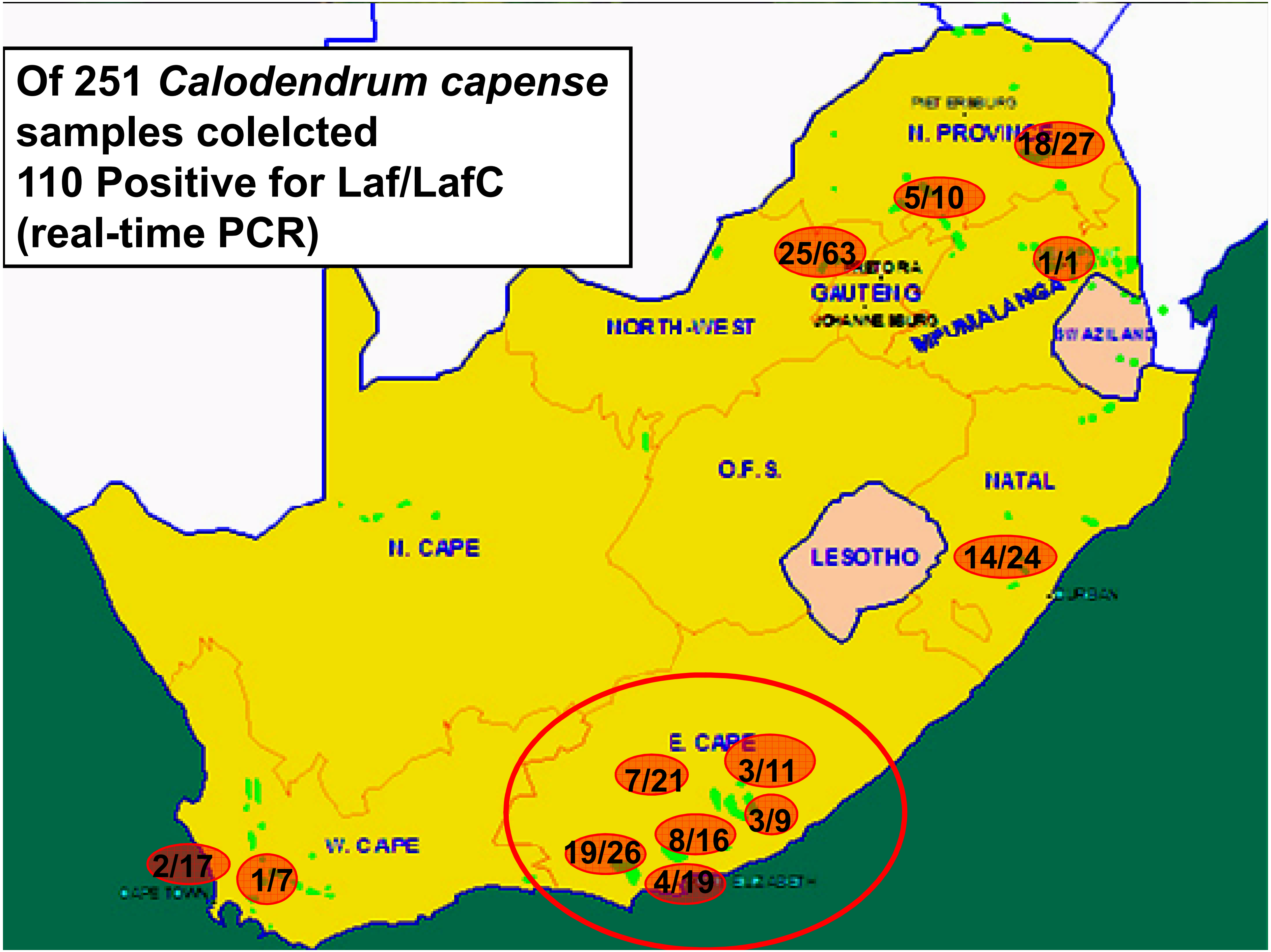
Collect 251 *Calodendrum capense* specimens from various sites in South Africa:

- **Indigenous vegetation surrounding citrus orchards,**
- **In botanical gardens**
- **In natural settings**

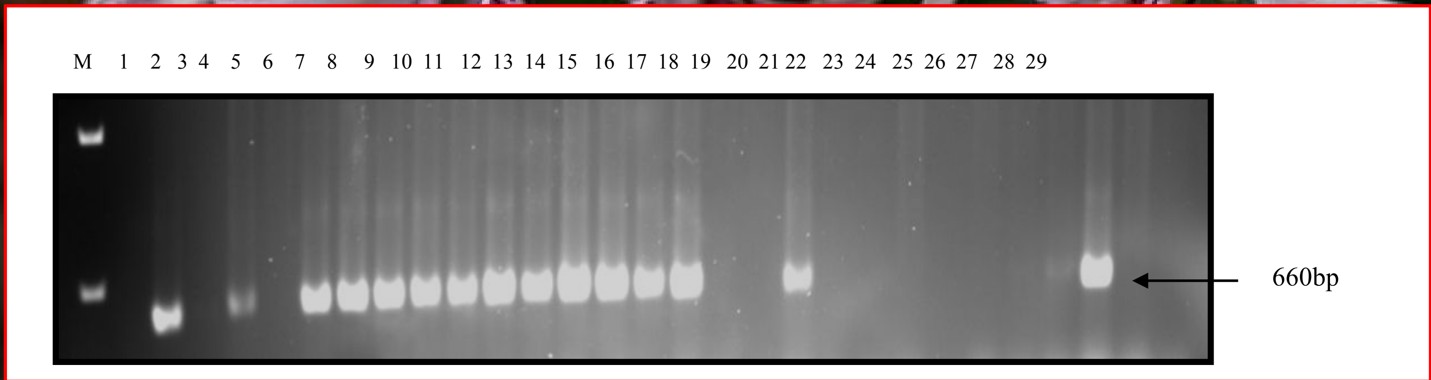
Survey 2006-2010.
Collect 251 *Calodendrum capense* samples



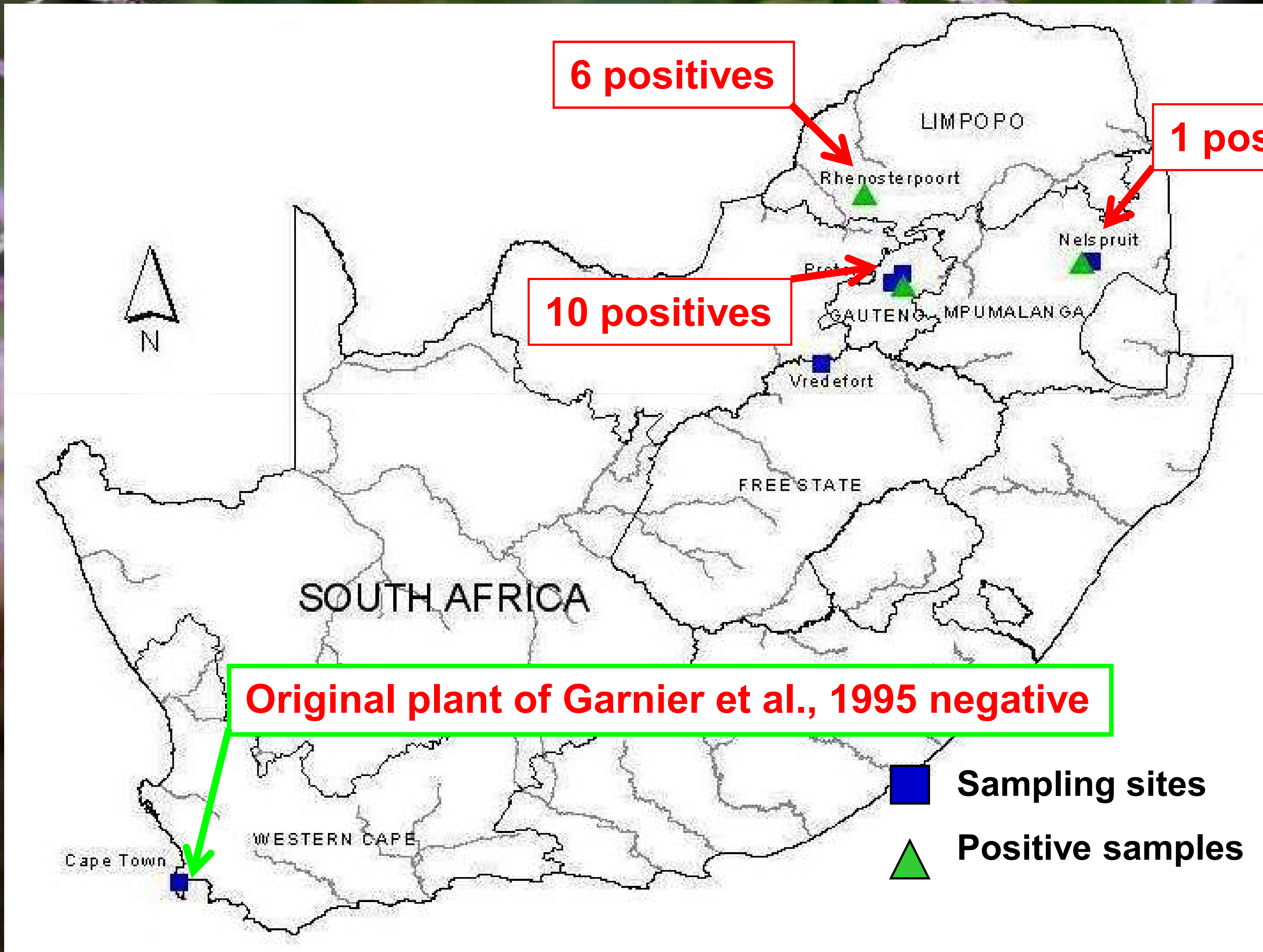
Of 251 *Calodendrum capense* samples colelcted
110 Positive for Laf/LafC
(real-time PCR)

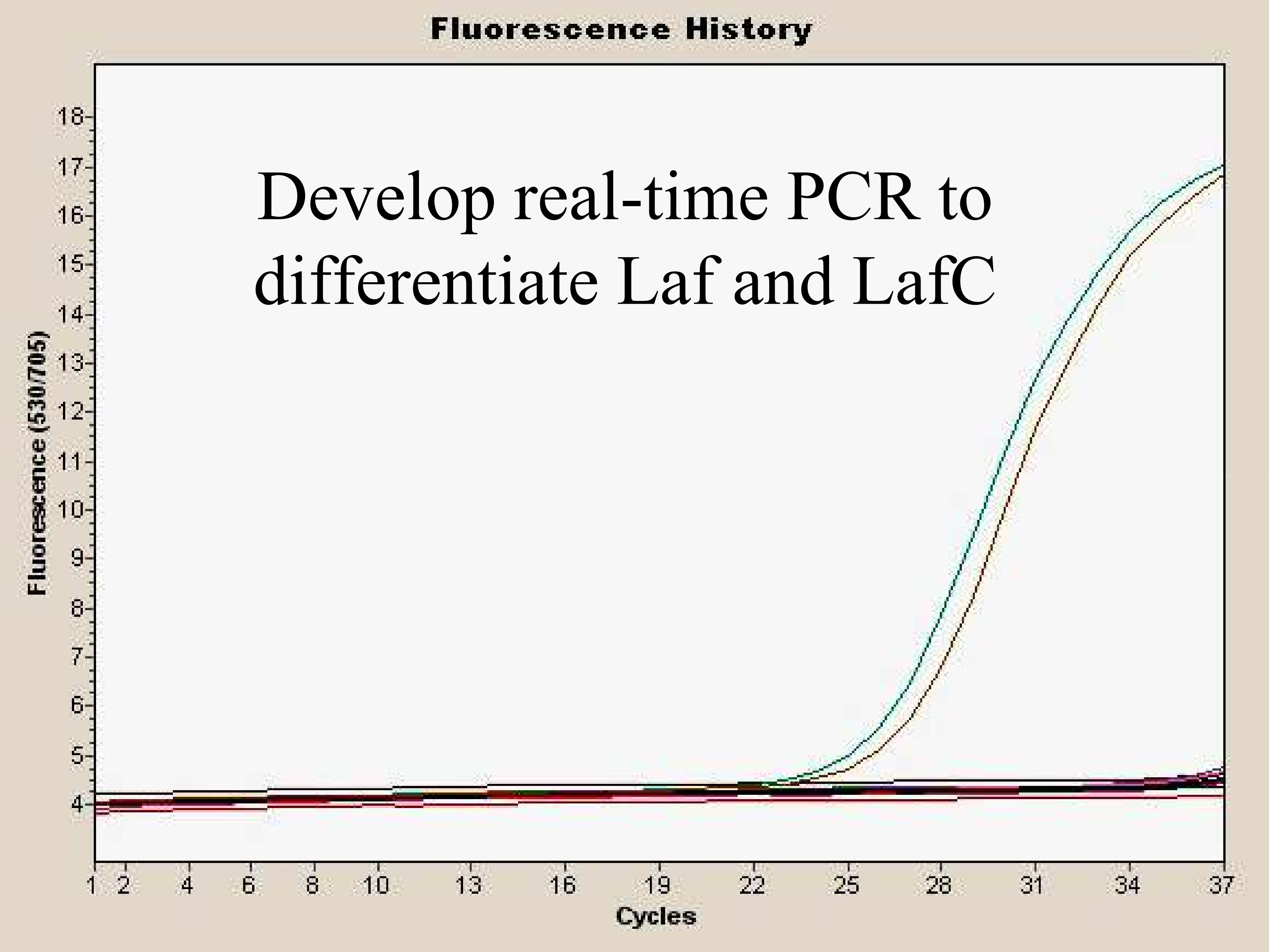


17 of the *Calodendrum capense* collected in the first year of the survey and yielding amplicons using the A2/J5 primer pair, were sequenced and have identities of 92 to 97% with “*Ca*. Liberibacter africanus subp. capensis”



Calodendrum capense positive samples sequenced




Fluorescence History
Develop real-time PCR to
differentiate Laf and LafC
Fluorescence (530/705)
18
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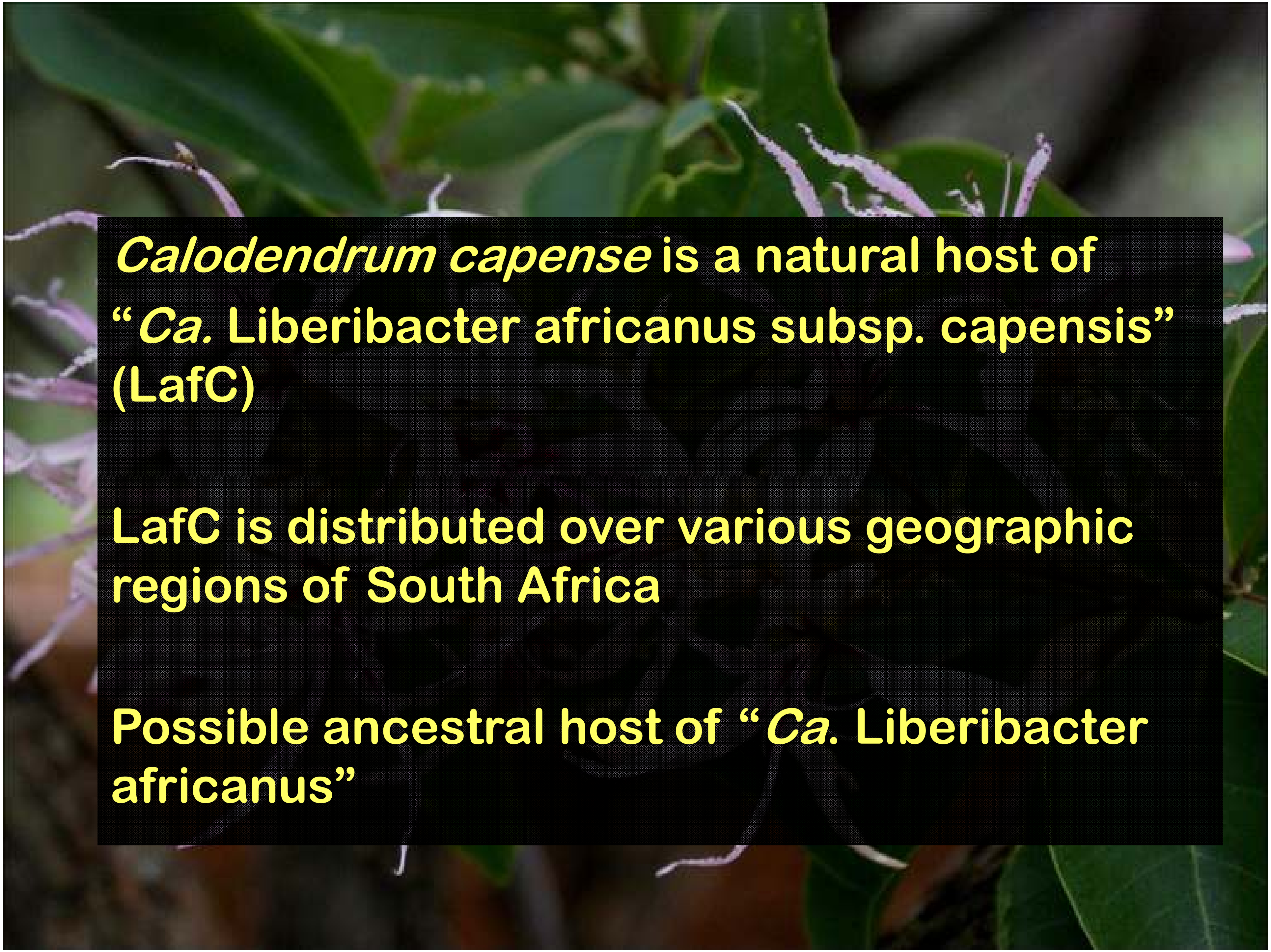
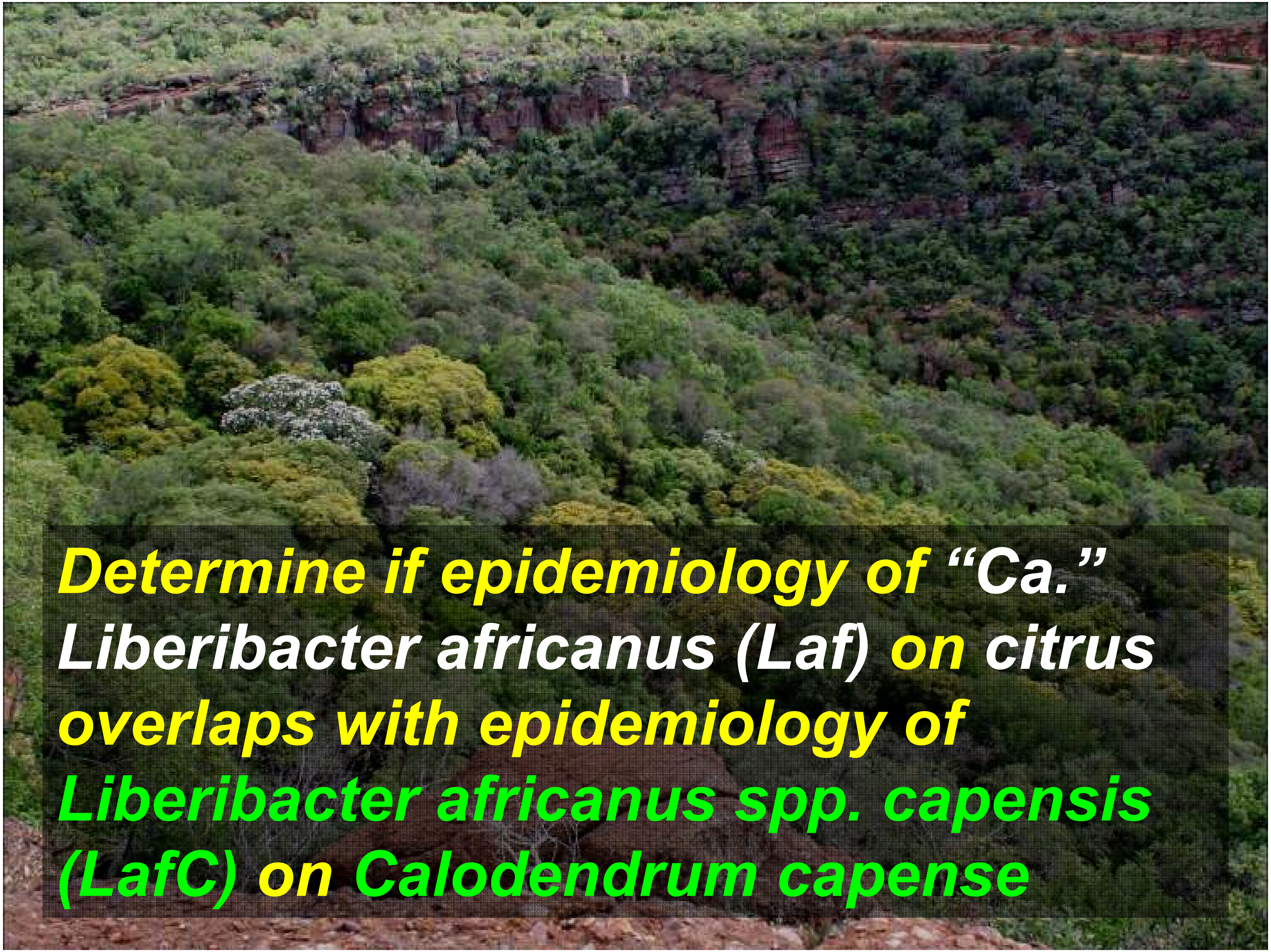
28
31
34
37
Cycles

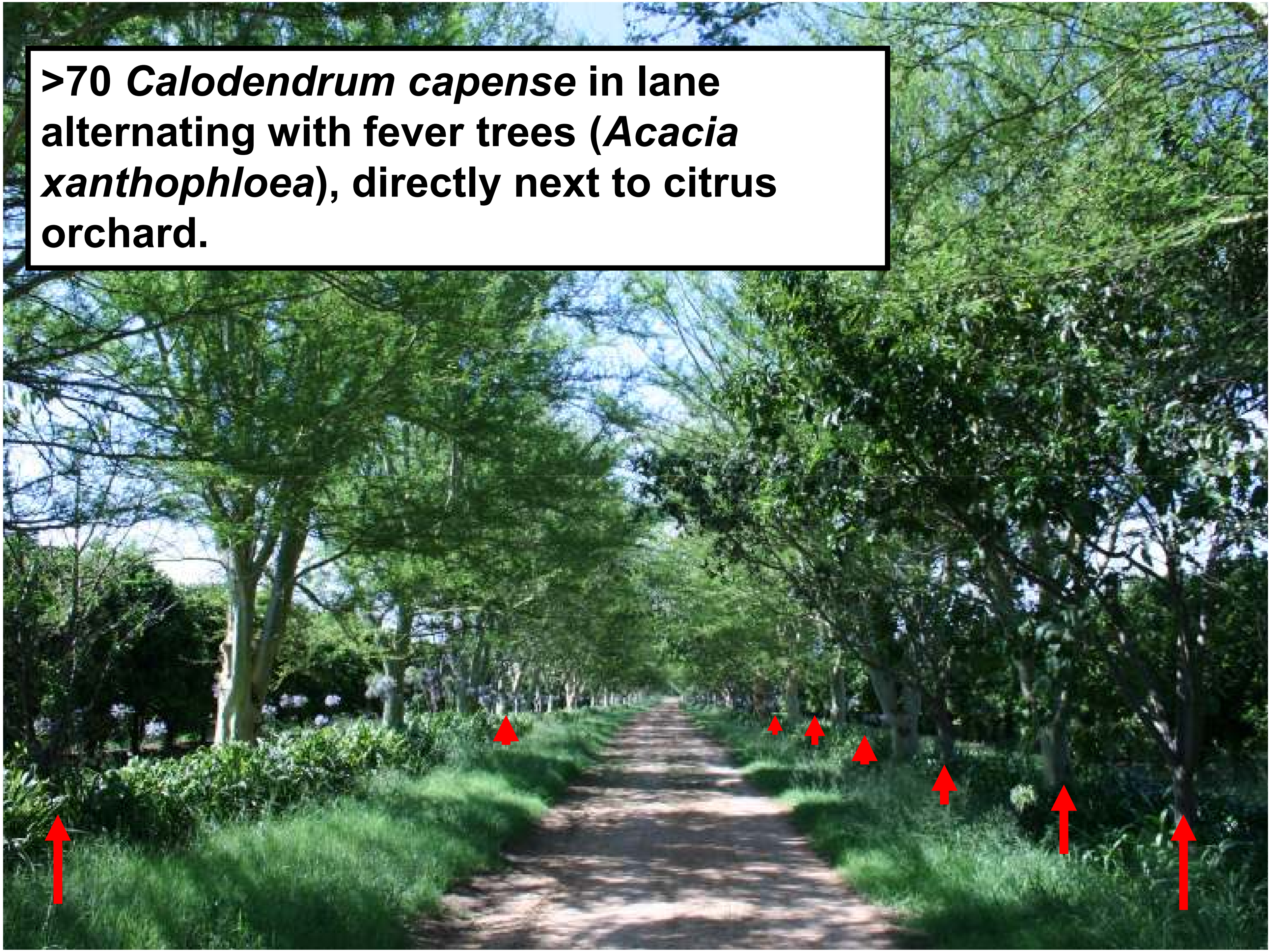


Calodendrum capense is a natural host of "*Ca.* Liberibacter africanus subsp. capensis" (LafC)

LafC is distributed over various geographic regions of South Africa

Possible ancestral host of "*Ca.* Liberibacter africanus"


Determine if epidemiology of "Ca." Liberibacter africanus (Laf) on citrus overlaps with epidemiology of Liberibacter africanus spp. capensis (LafC) on Calodendrum capense


>70 *Calodendrum capense* in lane alternating with fever trees (*Acacia xanthophloea*), directly next to citrus orchard.

Collect citrus plants directly adjacent the Calodendrum capense trees.

Test both species for Laf/LafC and CTV

Reciprocal transmission:

	Healthy Citrus	Healthy C. capense
LafC on C. capense		
Laf on citrus		

Grafting

Trioza erytreae

Summary: Greening in South Africa

"Ca" Liberibacter asiaticus and L. americanus
NOT in South Africa.

Only "Ca Liberibacter africanus" *<u>on citrus</u>* **in South Africa (no "Ca. L. africanus spp. capensis").**

Only "Ca. L. africanus spp. capensis" on indigenous Rutaceous hosts

Spread of *"Ca.* Liberibacter asiaticus" to mainland Africa.