



# Biology, background and worldwide situation of leprosis and its vector

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# Importance of leprosis in Brazil

- ❖ Leprosis: main citrus viral disease
- ❖ Vector present in orchards throughout the year
- ❖ Causes severe symptoms, particularly in sweet orange varieties

- ❖ Increase in production costs (pruning, anticipated renewal of the orchard...)
- ❖ Acaricides
  - US\$ 60 to 100 millions/ year
  - Environmental cost



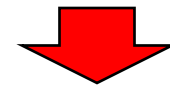
# Distribution in Brazil



# Distribution in the world

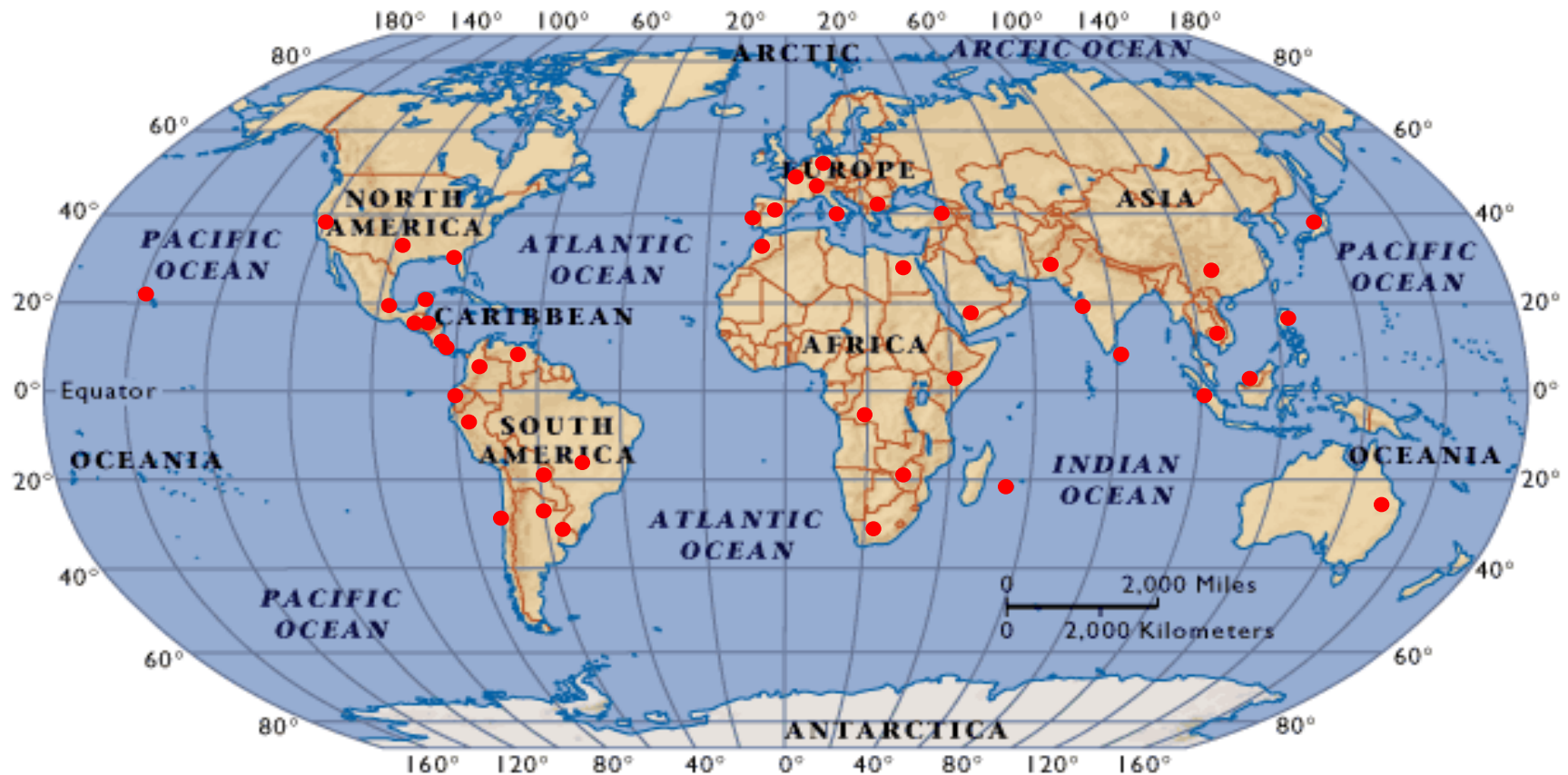


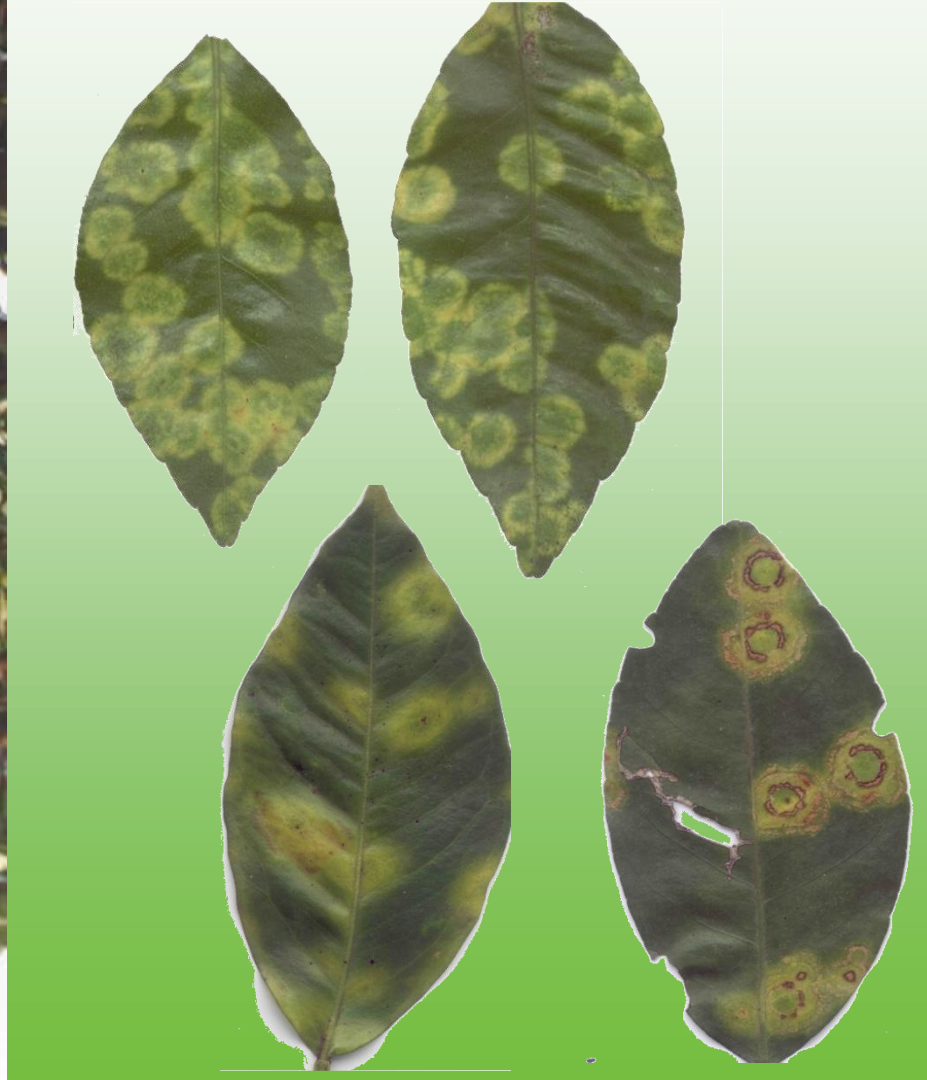
**Unconfirmed reports:**  
South of China, North of  
India, Sri Lanka, Japan,  
Philippines, Java, Egypt,  
and South Africa



**Typical American disease...**

... with a very cosmopolitan vector





















# History: etiology of the disease

The fungal hypothesis (Fawcett, Spegazzini)

Association with *Brevipalpus* mites (Frezzi,

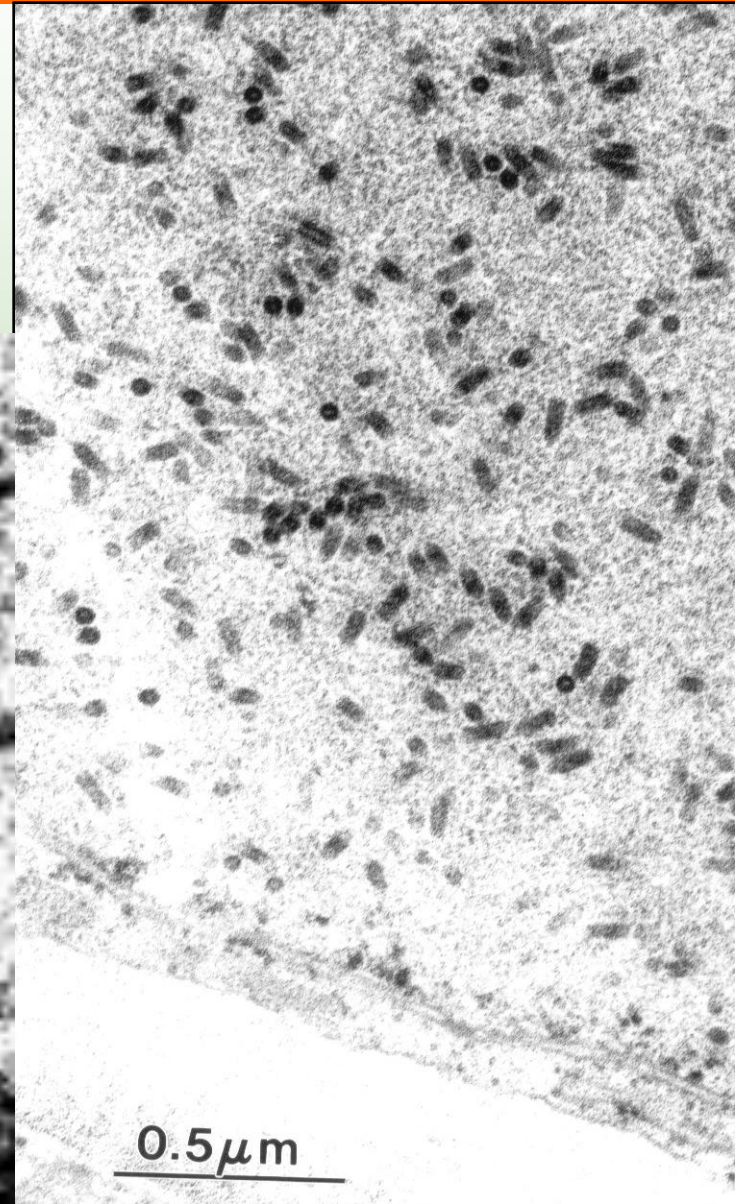
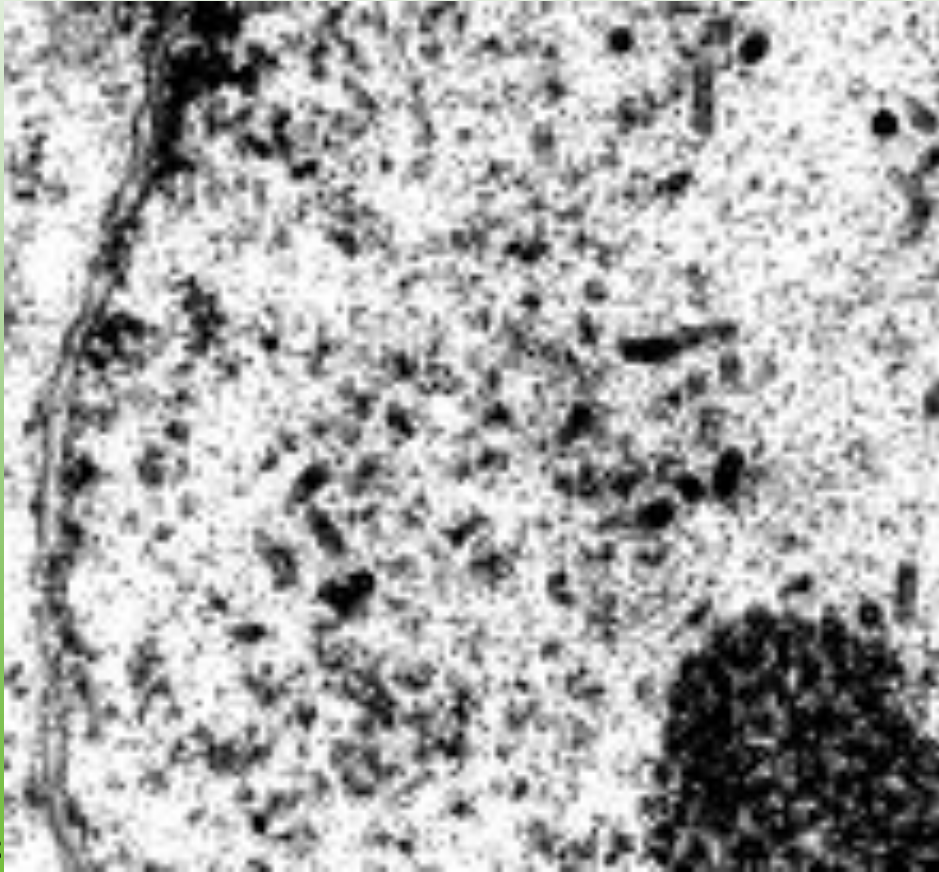
The mite-transmitted pathogen theory

- ringspot symptoms (Bitancourt)
- graft transmission (Knorr; Chagas & Rossetti)
- TEM visualization of particles (Kitajima et al.)
- mechanical transmission (Colariccio et al.)



# Citrus leprosis virus

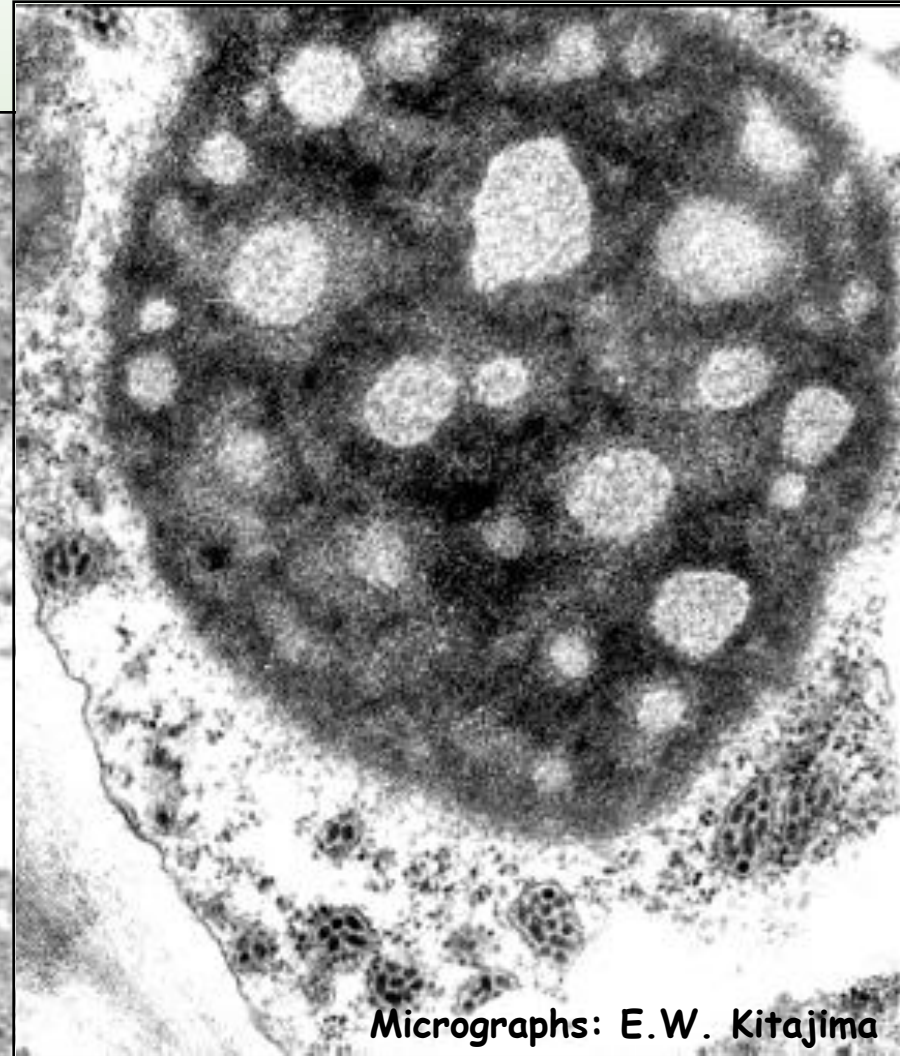
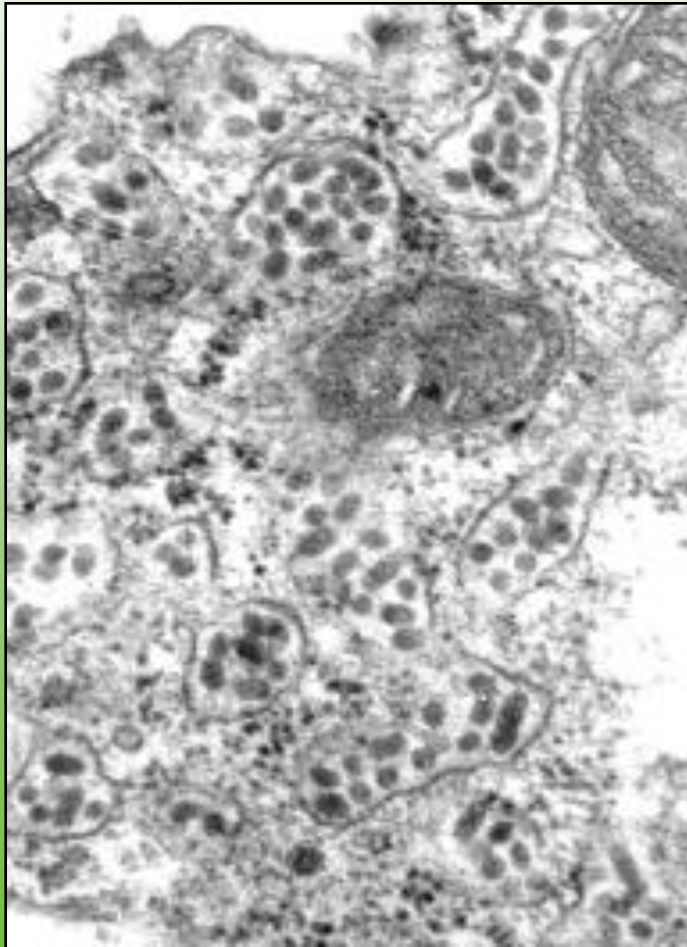
- \*Viral etiology
- Rhabdo-like particles in the nucleus



Micrographs:

# A second Citrus leprosis virus

- \*Rhabdo-like particles in the cytoplasm
- Electron-dense viroplasm



Micrographs: E.W. Kitajima

# One disease, two causal agents

Type	CiLV-C	CiLV-N
Morphology	bacilliform	bacilliform
Transmission	<i>Brevipalpus</i> sp.	<i>Brevipalpus</i> sp.
Particle size	50-60 × 100-120 nm	40-50 × 100-110 nm
Particle accum.	cytoplasm	nucleus
Symptoms	ClI, nII	ClI, nII
Occurrence	>99%	rare

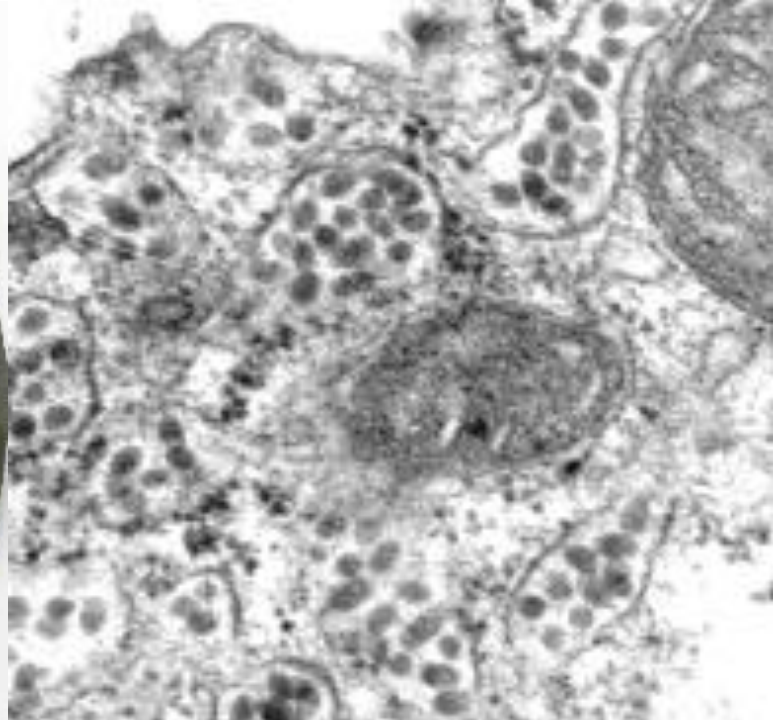


**In Brazil:** 1st report in 1972 (SP) and few other reports (SP, MG, RS)

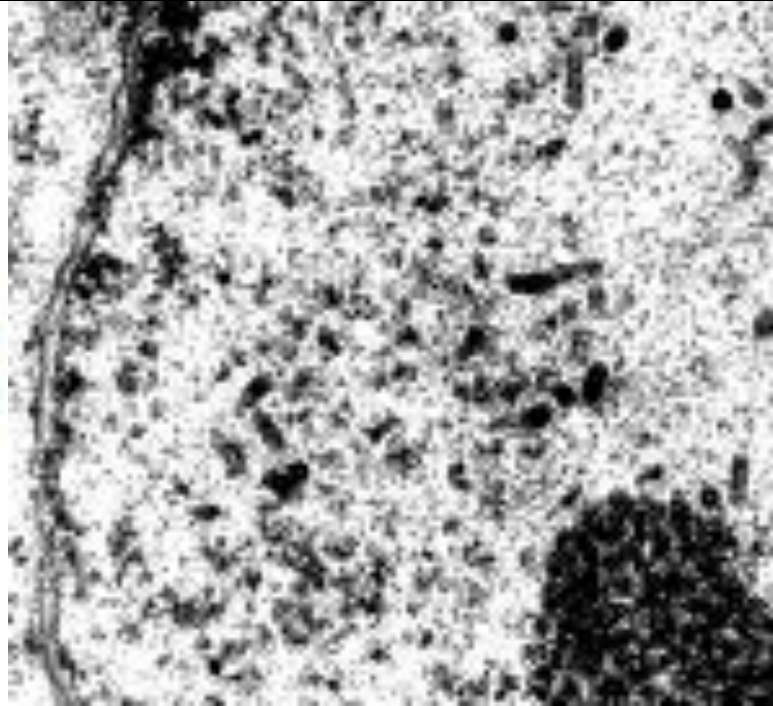
**In the world:** 1 report in Panamá (Boquete)

**Cooler climates, small orchards (backyards)**

CiLV-C

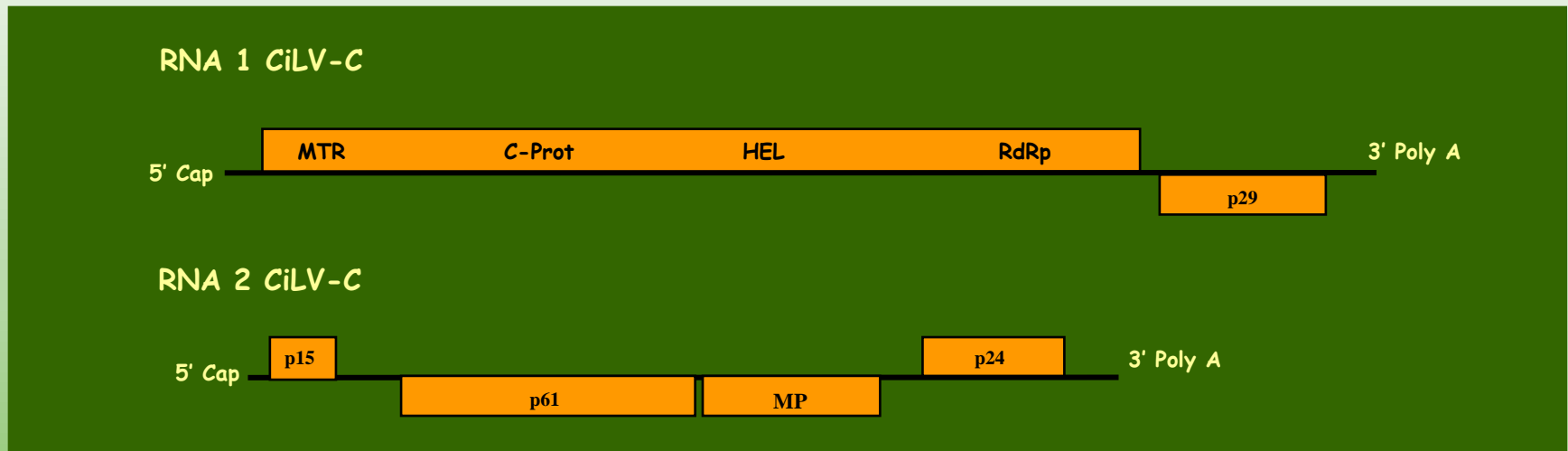


CiLV-N

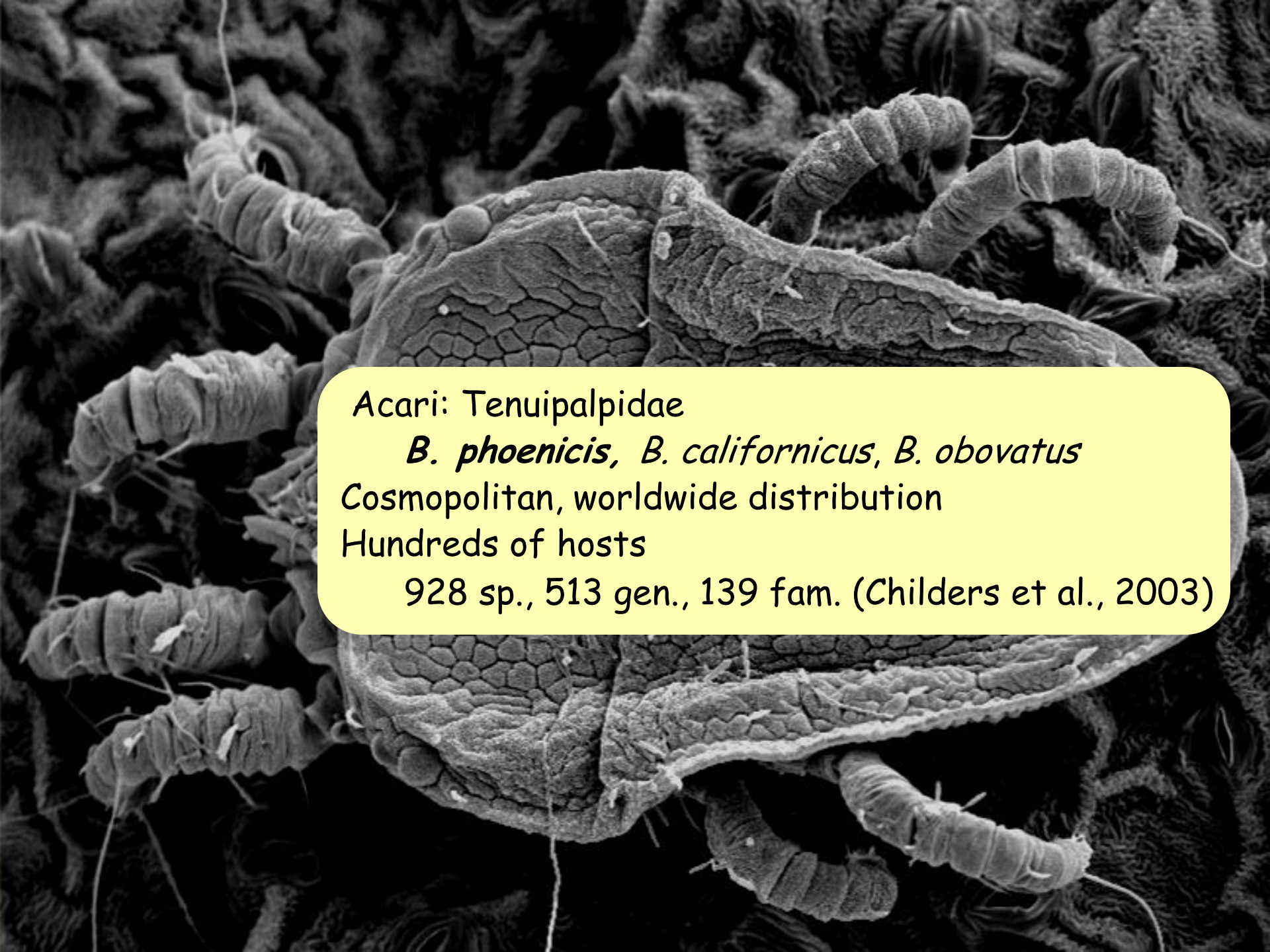


# Classification of the virus

Tentatively classified as members of the *Rhabdoviridae* family  
-morphology  
-cytoplasm x nucleus



Type member of the genus Cilevirus



Acari: Tenuipalpidae

*B. phoenicis*, *B. californicus*, *B. obovatus*

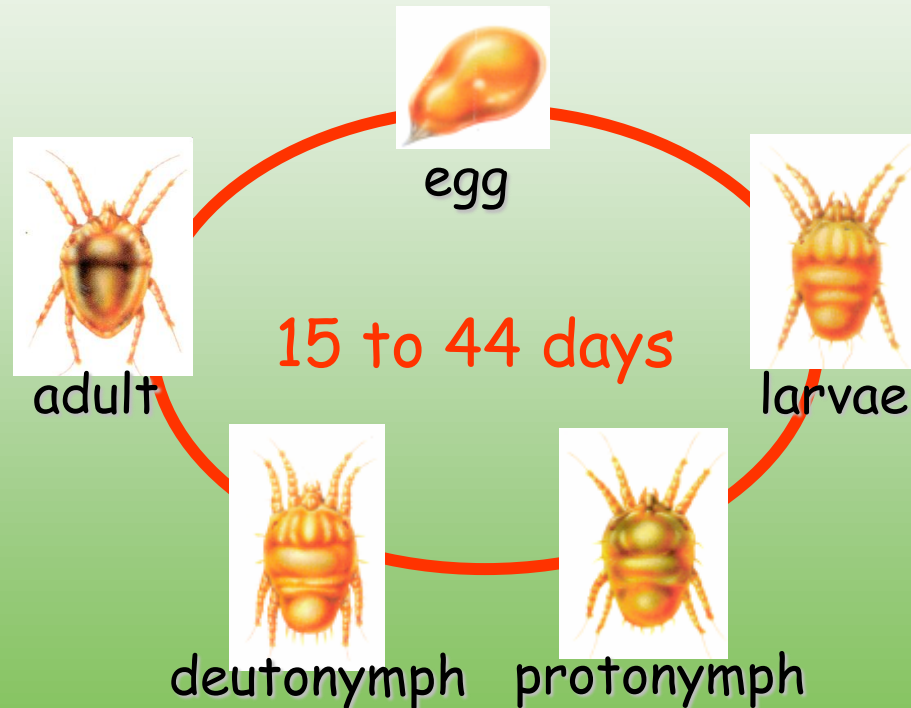
Cosmopolitan, worldwide distribution

Hundreds of hosts

928 sp., 513 gen., 139 fam. (Childers et al., 2003)

# Life cycle

Egg-adult: 15 to 44 days, at 30 and 20°C, respectively (whole cycle from 37 to 62 days)

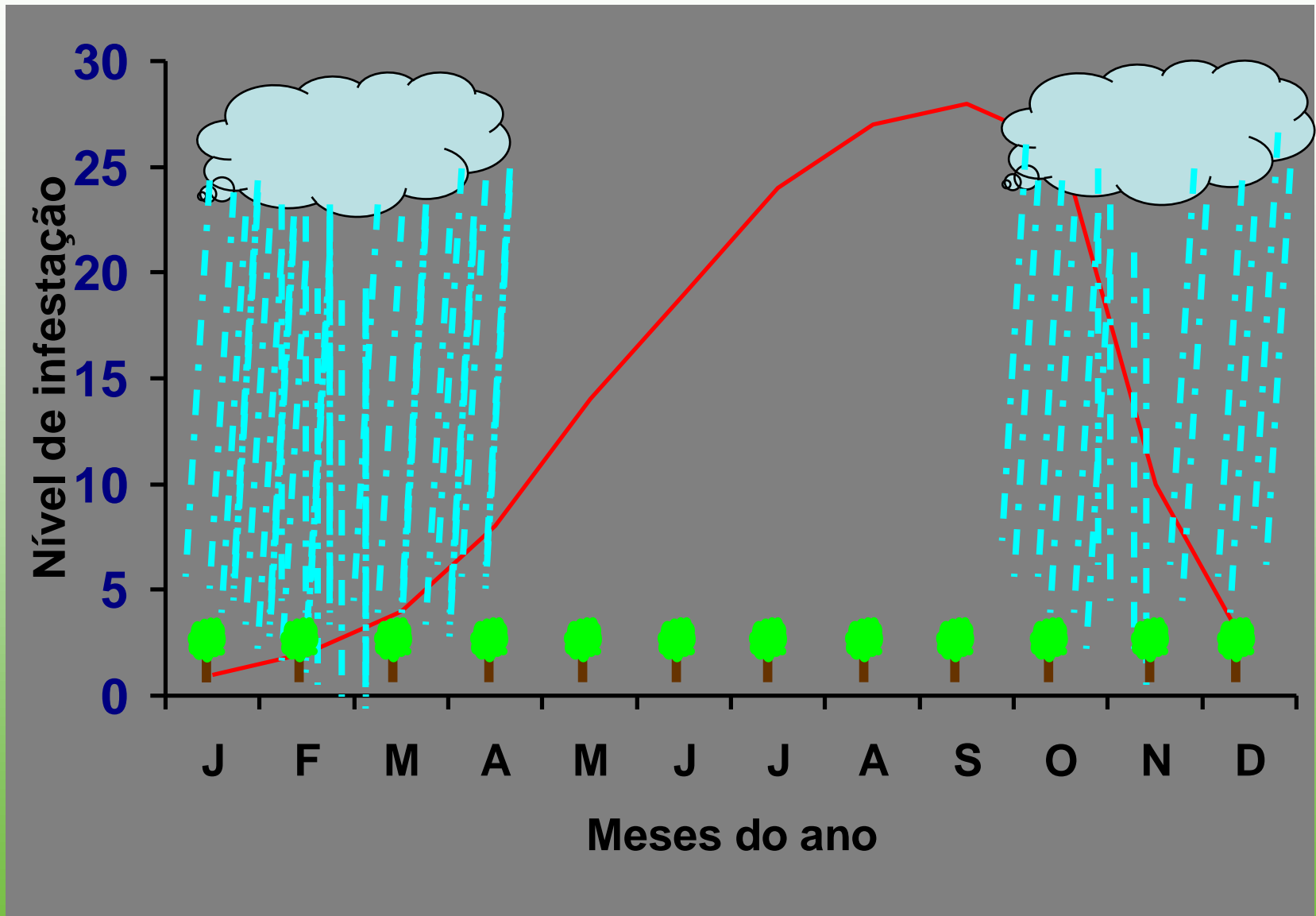


Often ~1 egg/day

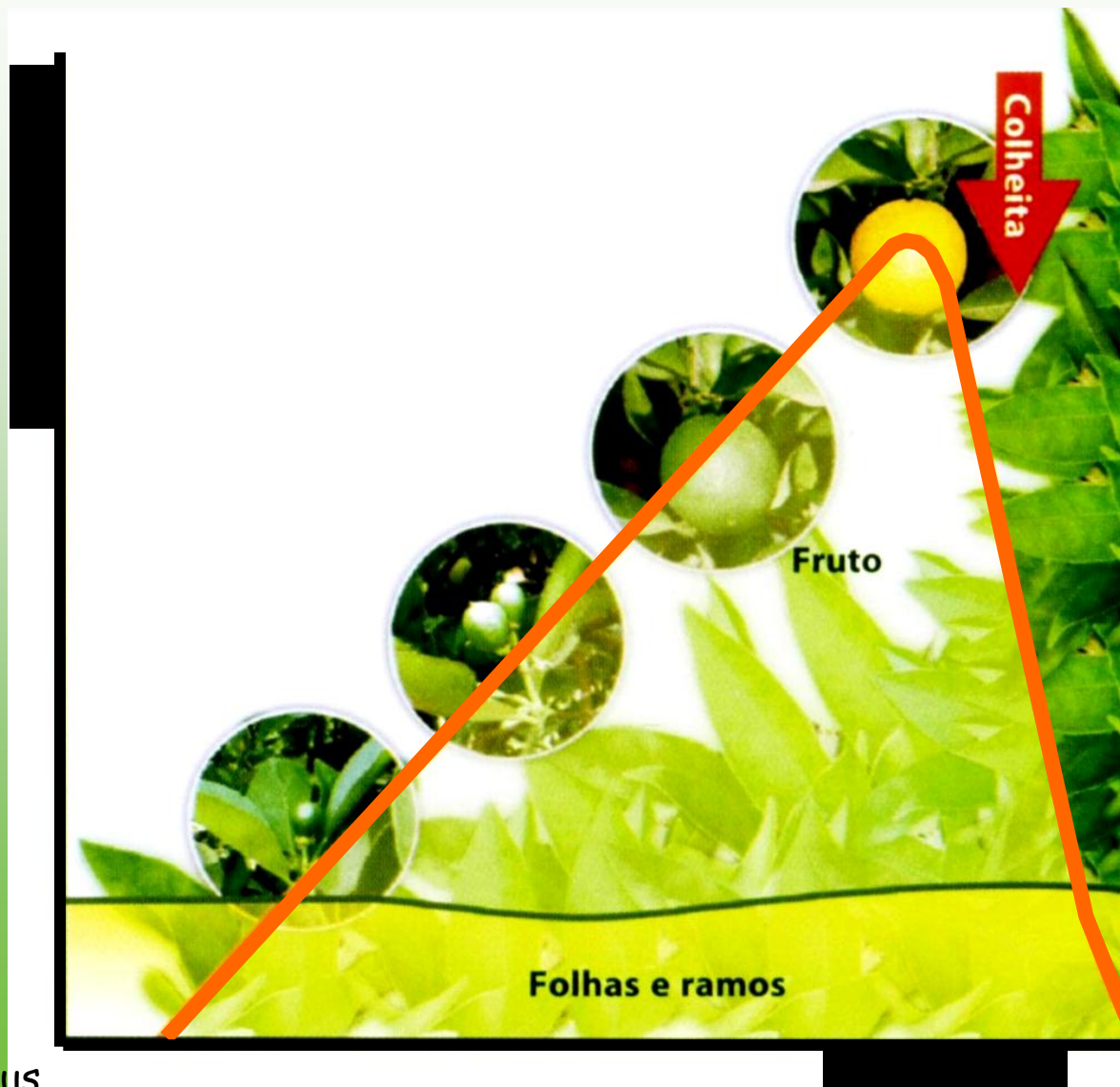
Sweet orange Pêra Rio at 30°C: 1.5-2 eggs/day

# Population fluctuation

Mites in orchards throughout the year (fruits > branches > leaves)



# Population X Development of the fruit



# Host range

## Sweet oranges

Some differences among varieties: Natal > Pêra > Valencia > Hamlin



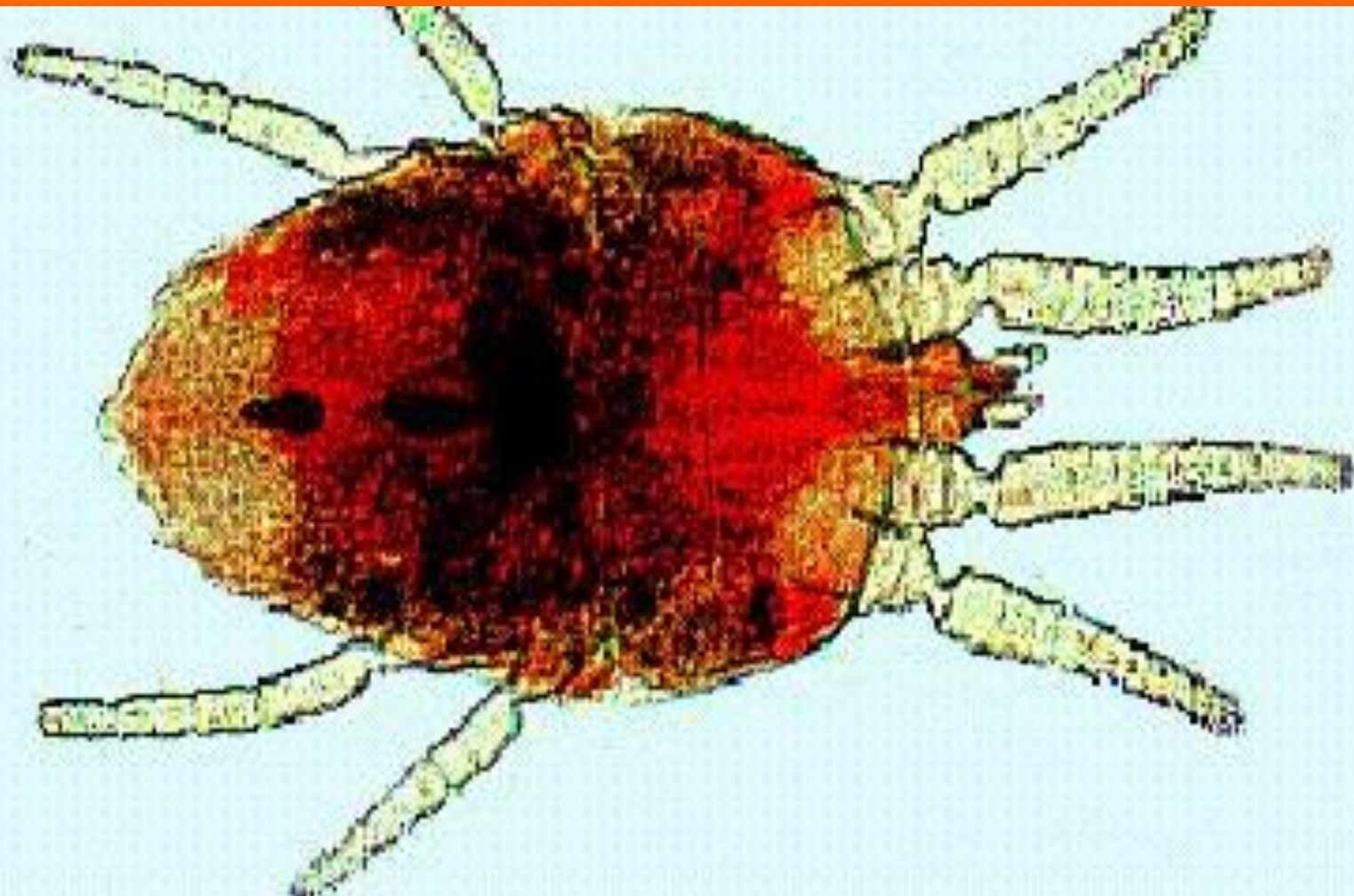
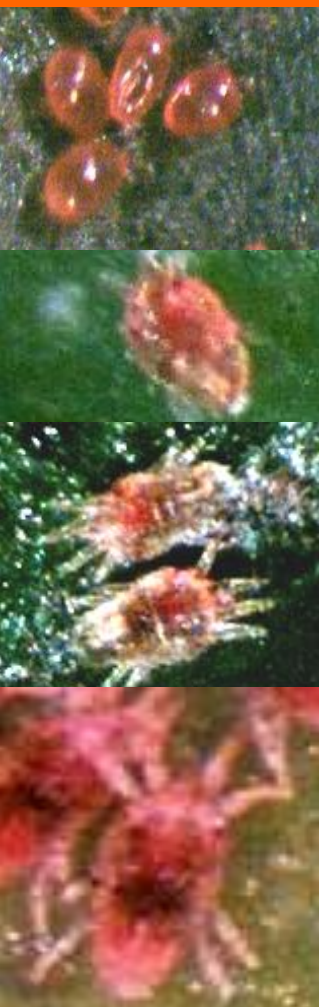
## Mandarins (rare fruit symptoms)

Cravo, Clementine Caçula 2, and Mogi das Cruzes mandarin

- Experimental host range include herbaceous, hedgerow and windbreak plants: *Solanum violaeifolium*, *Phaseolus vulgaris*, *Commelina* sp., *Malvaviscus arboreus*, *Glycosmis* sp., *Grevillea* sp. (Nunes, 2006)
- Natural host: *Swinglea glutinosa* (León et al., 2008)



# Transmission biology



- All active stages transmit (Chagas et al., 1984)
- Transovarial transmission (Knorr, 1968)
- Vector-virus interaction: persistent propagative (Rodrigues, 2001)

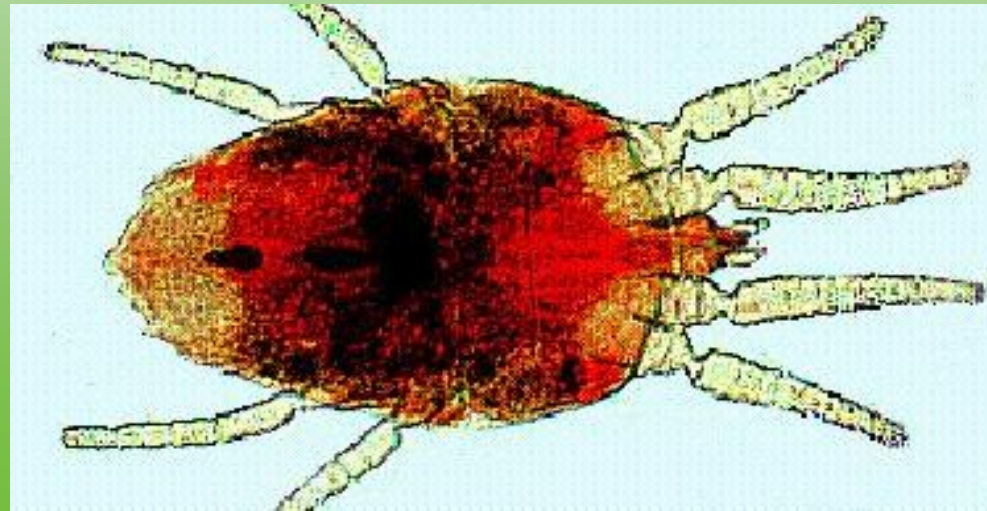
# *CiLV-Brevipalpus* interaction

- The virus is transmitted by all of the active stages
  - Larvae: 48.3%; Nymphs: 8.7%; **Adults: 7.6%** (Chagas et al., 1984)

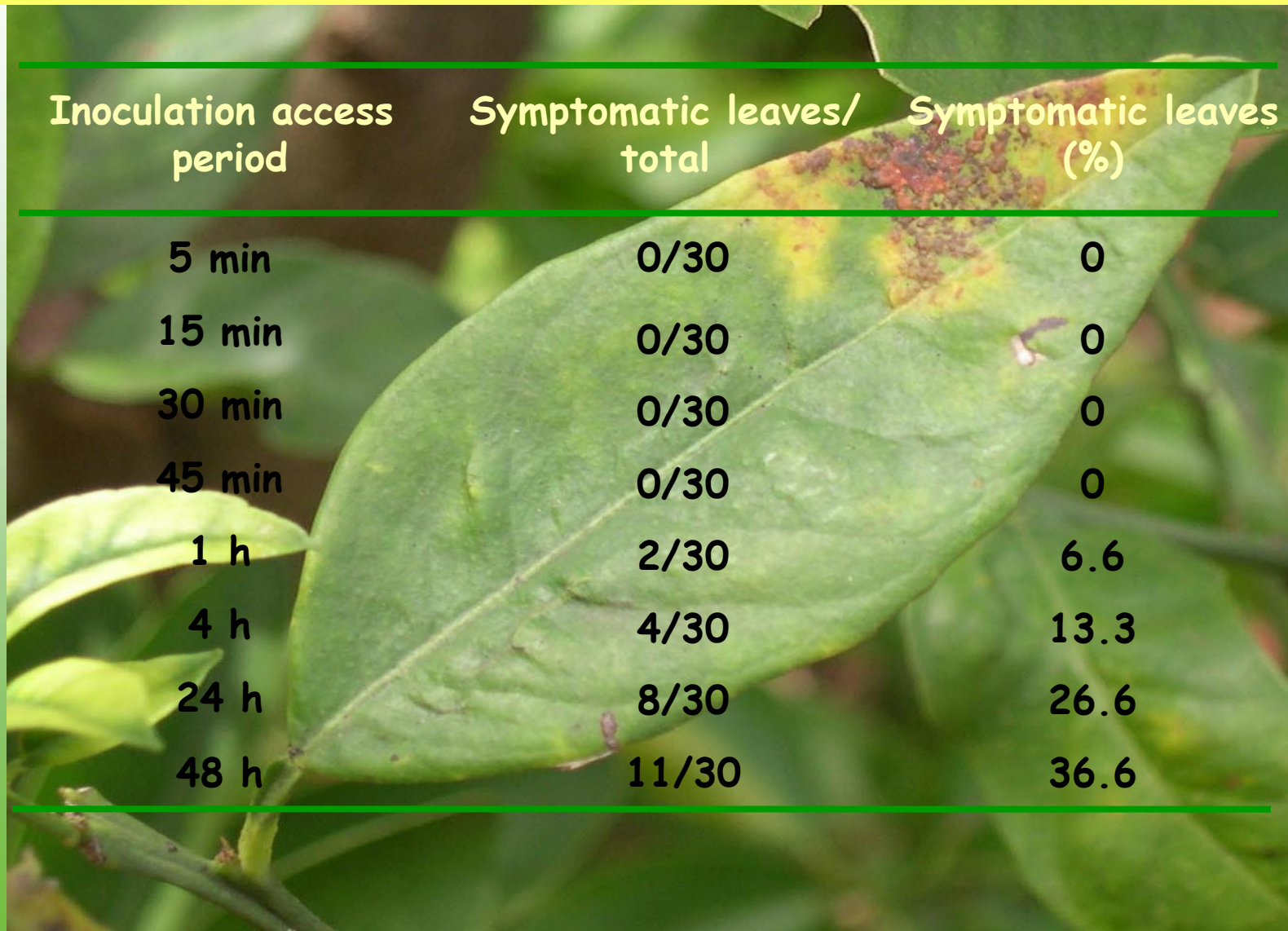


# *CiLV-Brevipalpus* interaction

Acquisition access period	Symptomatic leaves/ total	Symptomatic leaves (%)
1 h	0/30	0
4 h	1/30	3.3
24 h	6/30	20.0
48 h	6/30	20.0



# *CiLV-Brevipalpus* interaction



Inoculation access period	Symptomatic leaves/ total	Symptomatic leaves (%)
5 min	0/30	0
15 min	0/30	0
30 min	0/30	0
45 min	0/30	0
1 h	2/30	6.6
4 h	4/30	13.3
24 h	8/30	26.6
48 h	11/30	36.6

# *CiLV-Brevipalpus* interaction

Mite	Longevity (days)	Number of eggs/ day
Non-viruliferous	27	1.12
Viruliferous	20	1.16

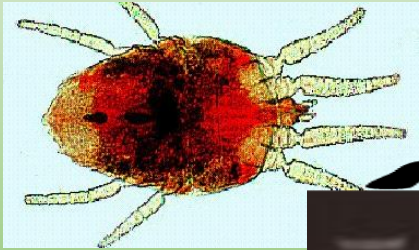
# CiLV-*Brevipalpus* interaction

## - Transovarial transmission?

*B. obovatus* and *B. californicus* in Florida (Knorr, 1968)

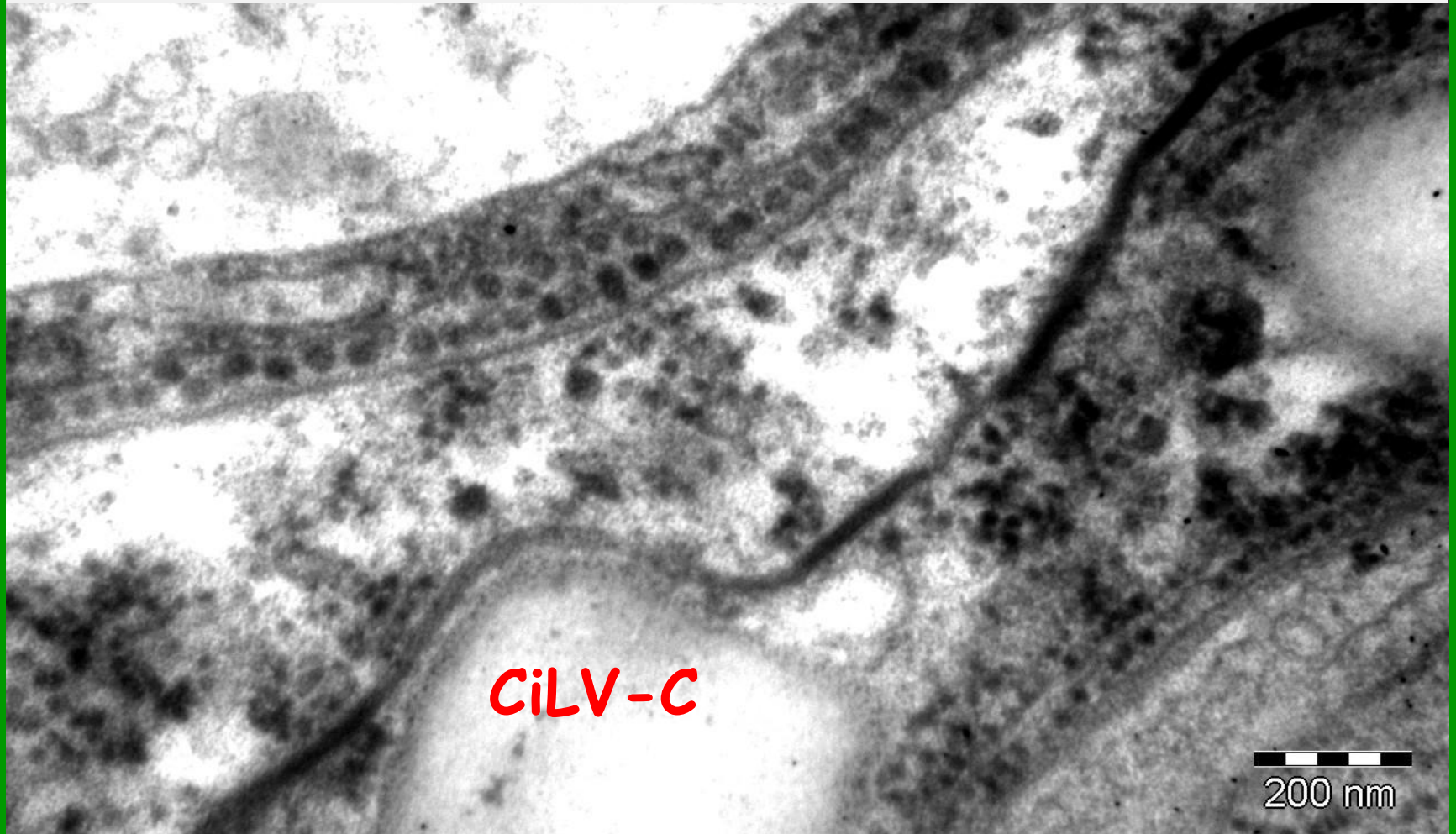
X

*B. phoenicis* (Boaretto et al., 1993; Novelli et al., 2005)



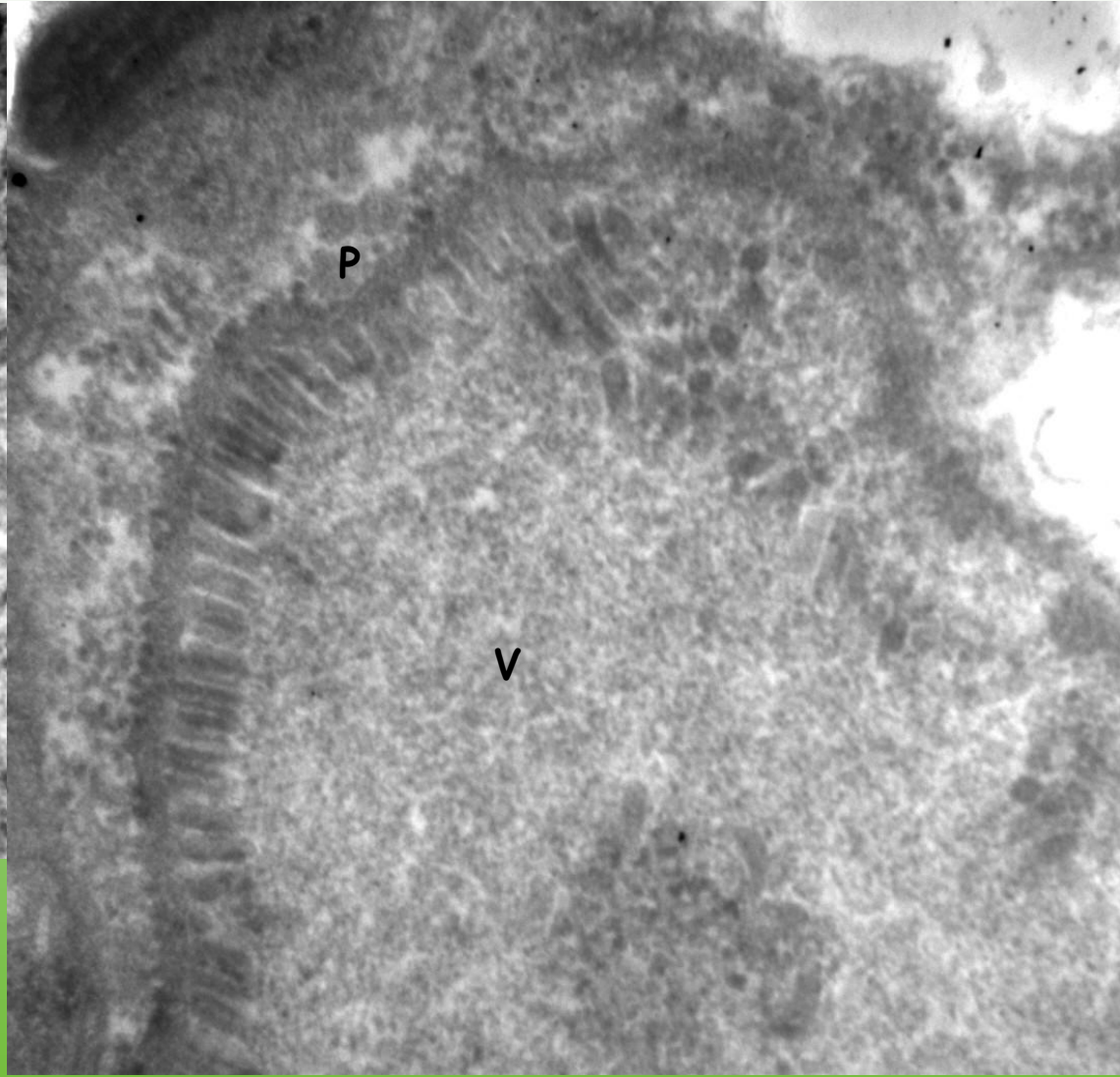
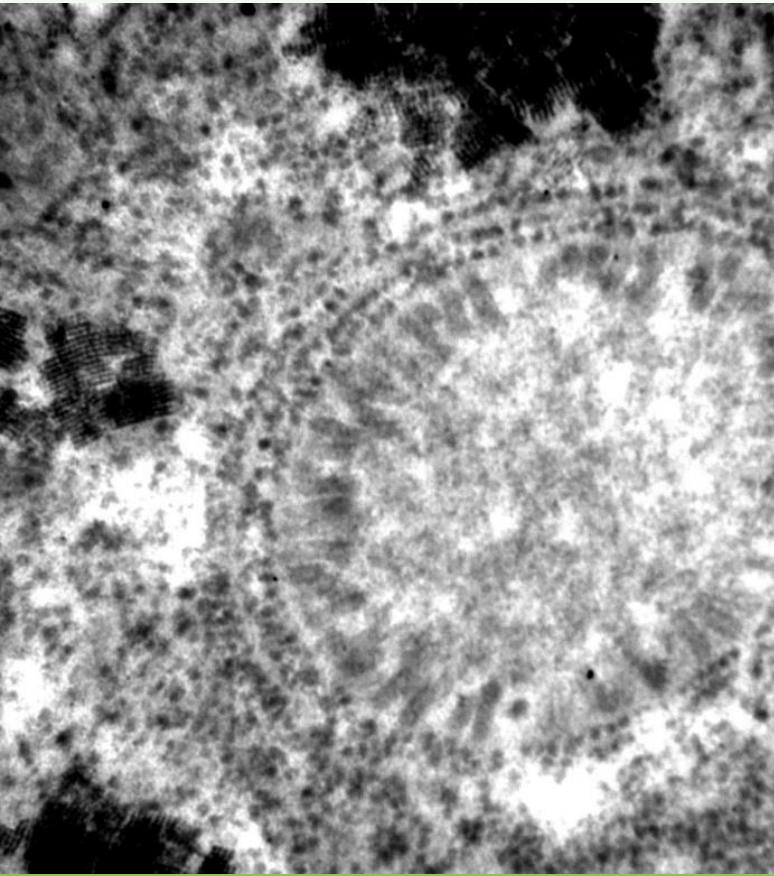
M = 1 Kb ladder; 1, 10, 11= eggs; 2, 4, 7, 9= adults; 3, 5, 8= larvae; 6= nymphs; 12= non-viruliferous mites

- Hundreds of viruliferous mites analyzed by TEM
- Few particles observed
- Presumed virions between adjacent to membranes near the intestine
- Absence of viroplams



## CiLV-N

- Spoked wheel structure in cells of the prosomal gland (~salivary gland)
- Presumed particles in the nucleus, with viroplasm, or in the cytoplasm



# Quantification of the virus in the vector

Viruliferous mites transferred to:

- A) Symptomatic sweet orange;
- B) Rangpur lime

Samples were collected every two days for 30 days



qRT-PCR



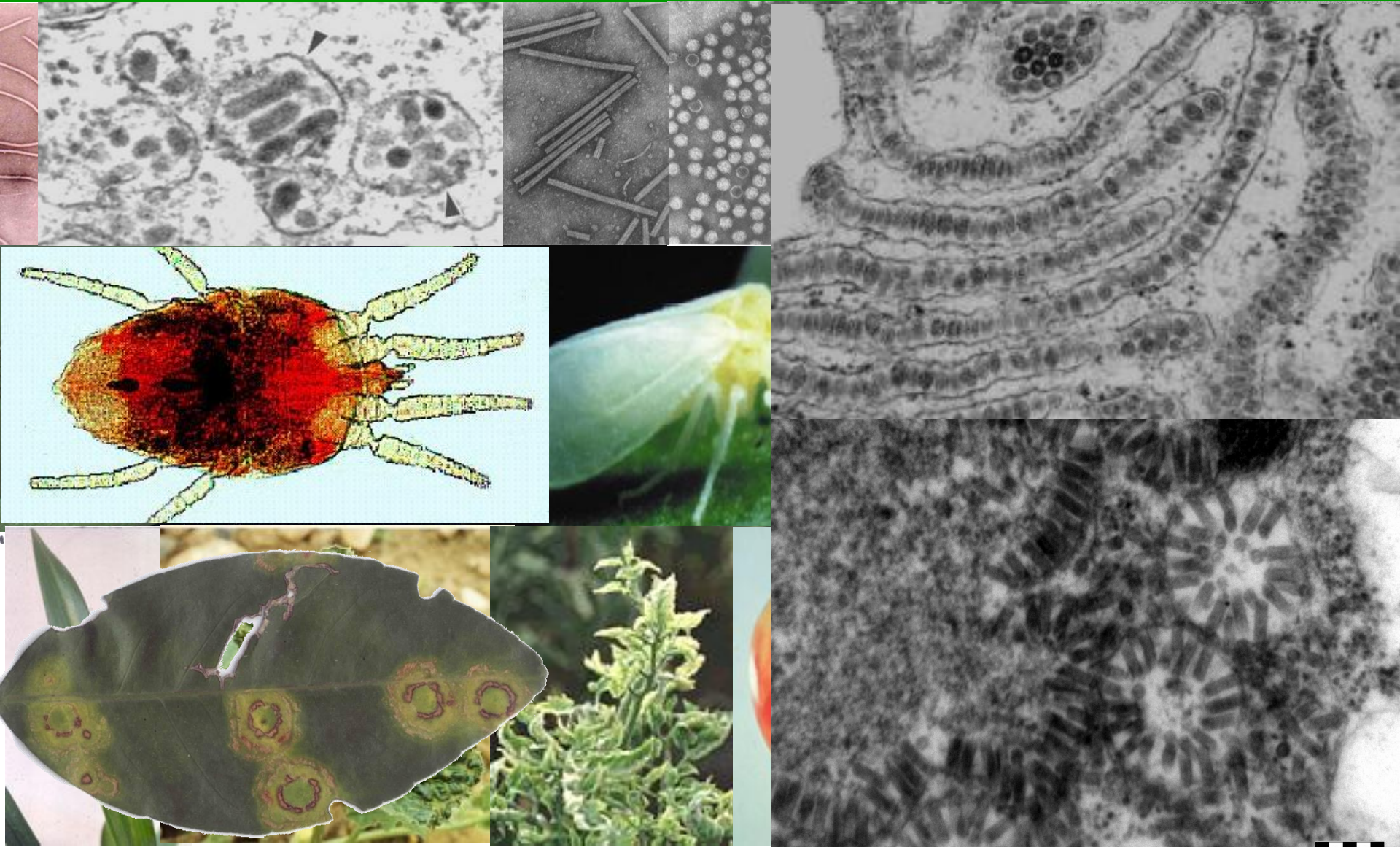
No differences in viral titer

# Transmission biology

The virus-vector relationship is of **circulative** (and not propagative) type

- Biological data on transmission
- RT-qPCR
- TEM

# CiLV x plant viruses



# More surprises...

Several of the "truths" about leprosis are now shown to be incorrect

- \* CiLV-C and CiLV-N: completely different viruses

- \* C

- \* H

- \* C

Unusual and challenging disease

e

- \* The virus is not a mite virus that is able to colonize plants





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