



# **Examination of host responses of different citrus varieties and relatives to HLB infection**

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# HLB host range

## Greenhouse experiments



under  
'select agent'  
conditions



# Main objectives in this study:

- 1) Examine different citrus genotypes or citrus relatives with the purpose of understanding at the whole plant level how different citrus varieties respond to Las, and whether there are resistant or tolerant varieties.
- 2) Understand whether there is a correlation between severity of disease and the level of bacterial concentration
- 3) Examine how Las is distributed in infected plant, and where through out the infected host the bacterium is present at the highest concentration
- 4) Determine how environmental conditions affect symptoms development

# Citrus varieties and relatives that have been screened

- 1 Citrus macrophylla (Alemow)
- 2 Valencia Sweet orange
- 3 Ruby Red grapefruit
- 4 Nules clementine (mandarin)
- 5 Minneola tangelo
- 6 Eureka lemon
- 7 Volkamer lemon
- 8 Persian lime
- 9 Mexican lime
- 10 Citron
- 11 Carrizo citrange
- 12 Poncirus trifoliata
- 13 Citrus halimii
- 14 Citrus indica
- 15 Citrus micrantha
- 16 Citrus ambyllocarpa
- 17 Sour orange
- 18 Meiwa kumquat
- 19 Calamondin
- 20 Citrus hystrix
- 21 Swingle citrumelo
- 22 Sun Chu Sha (mandarin)
- 23 Palestine Sweet lime
- 24 Citrus latipes
- 25 Cleopatra mandarin
- 26 Madam vinous Sweet Orange
- 27 Duncan grapefruit
- 28 Ling Ping Yau pummelo
- 29 Hirado Buntan Pink pummelo
- 30 Siamese Sweet pummelo
- 31 Severinia buxifolia

We have not found citrus varieties or relatives that are resistant to infection by *Candidatus Liberibacter asiaticus*.

However, there were major differences in host response between different varieties.



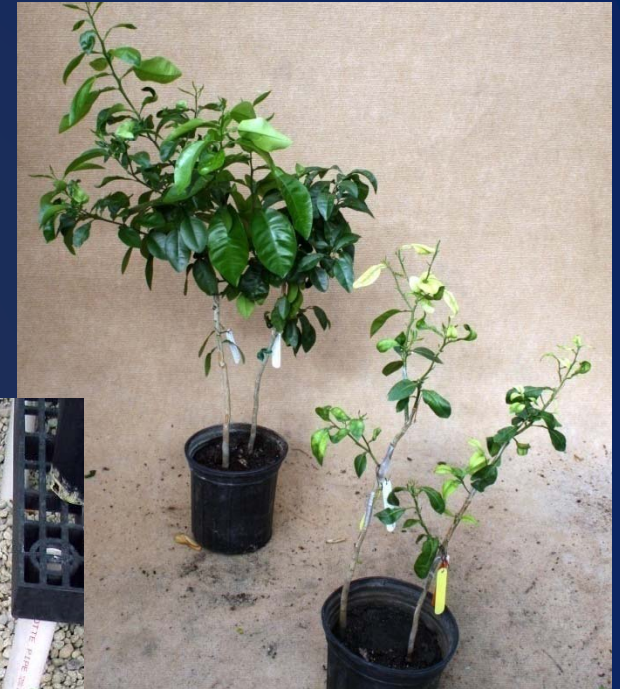
# Different citrus varieties have different degrees of susceptibility to HLB



Eureka lemon

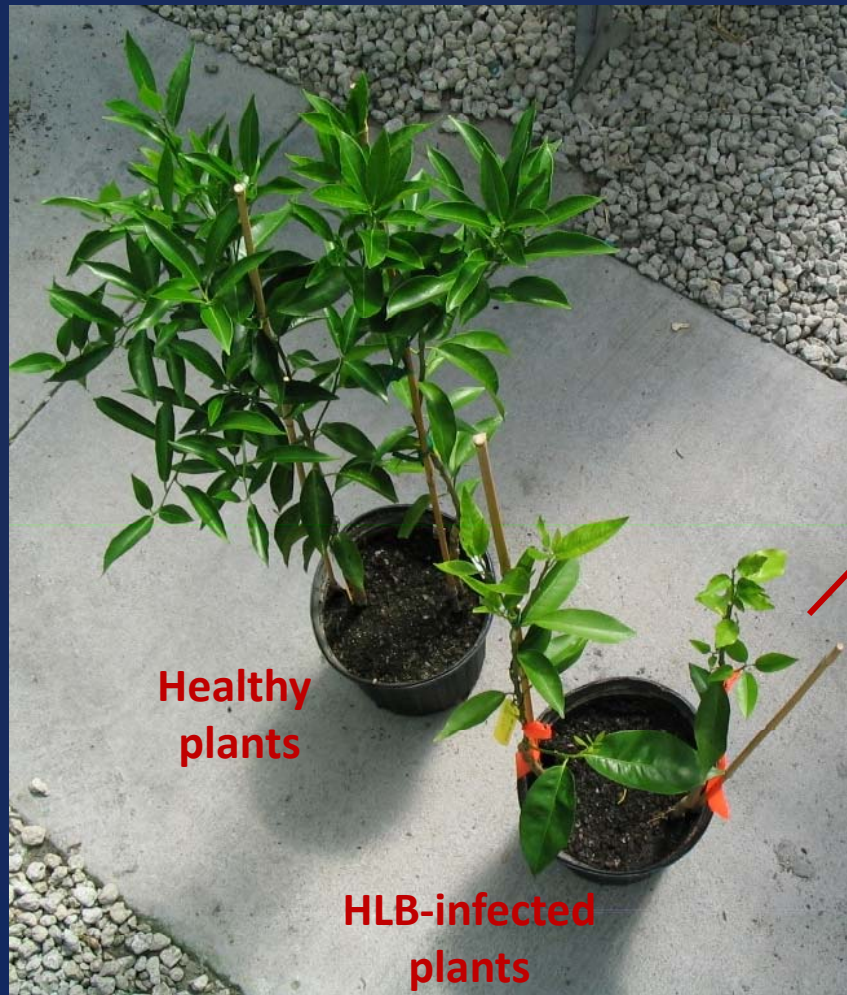


Cleopatra mandarin



Duncan grapefruit

# Nules clementine: high sensitivity to HLB



3 months upon inoculation



6 months upon inoculation



# Effect of HLB on Madam Vinous sweet orange (9 months after inoculation)





# Symptoms produced by different citrus varieties under greenhouse conditions



Eureka lemon



Mexican lime



Duncan grapefruit



Hirado Buntan pink pummelo



Madam Vinous sweet orange

# Response of different citrus genotypes to HLB under greenhouse conditions

Citrus or relative	Las titer	Symptoms description
<i>Poncirus trifoliata</i>	+	<b>Extreme tolerance</b> some stunting
Carrizo citrange	+	<b>Extreme tolerance</b> little or no chlorosis
Eureka lemon	++	<b>Extreme tolerance</b> chlorosis only under high light
Persian lime	+++	<b>Extreme tolerance</b> no distinct symptoms

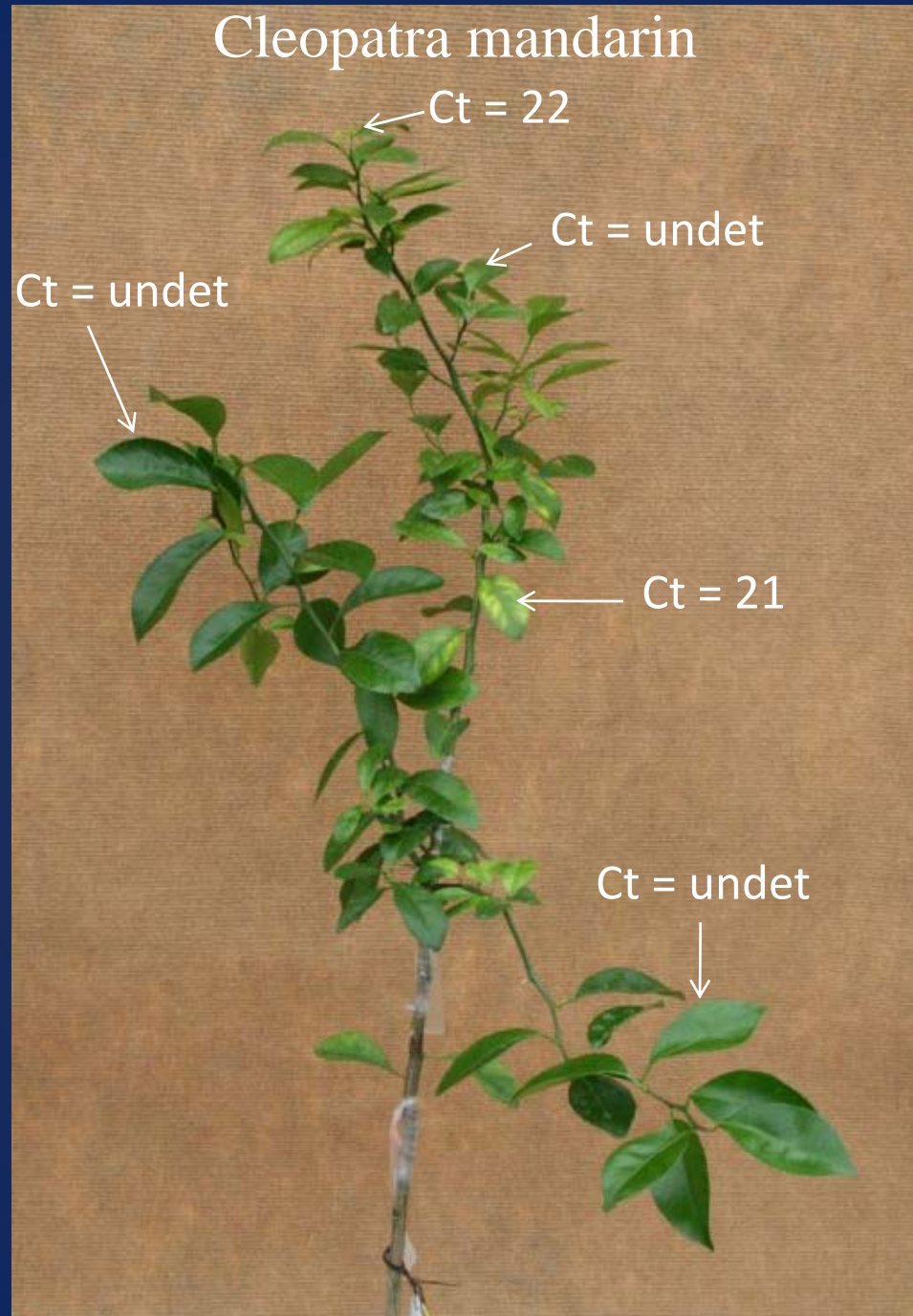
Sun Chu Cha	+++	<b>Tolerant:</b> scattered groups of chlorotic leaves
Sour orange	+++	<b>Tolerant</b>
Volkamer lemon	+++	<b>Tolerant</b>
<i>C. macrophylla</i>	+++	<b>Tolerant</b>
Swingle citrumelo	+++	<b>Tolerant</b>
Citron	++	<b>Tolerant</b>
Palestine sweet lime	+++	<b>Tolerant</b>
Mexican lime	+++	<b>Tolerant</b>
Calamondin	+++	<b>Tolerant</b>
<i>C. micantra</i>	+++	<b>Tolerant</b>



Siamese sweet pummelo	++	<b>Intermediate:</b> chlorotic leaves, some growth reduction
Ling Ping Yau pummelo	++	<b>Intermediate</b>
Hirado Buntan pink pummelo	++	<b>Intermediate</b>
<i>C. ambyllocarpa</i>	+++	<b>Intermediate</b>
Cleopatra mandarin	+++	<b>Intermediate</b>
<i>C. indica</i>	++	<b>Intermediate</b>
Meiwa kumquat	++	<b>Intermediate</b>

Valencia sweet orange	+++	<b>Sensitive:</b> chlorosis including tip, reduced growth
Madam Vinous sweet orange	+++	<b>Sensitive</b> - same
Duncan grapefruit	++	<b>Sensitive</b> - same
Ruby Red grapefruit	++	<b>Sensitive</b> - same
<i>Citrus halimii</i>	++	<b>Sensitive:</b> chlorosis, vein corking, death
Nules clementine	++	<b>Sensitive:</b> strong chlorosis, reduced growth, death
Minneola tangelo	++	<b>Sensitive:</b> strong chlorosis, reduced growth

Liberibacter  
often is unevenly  
distributed in  
infected trees





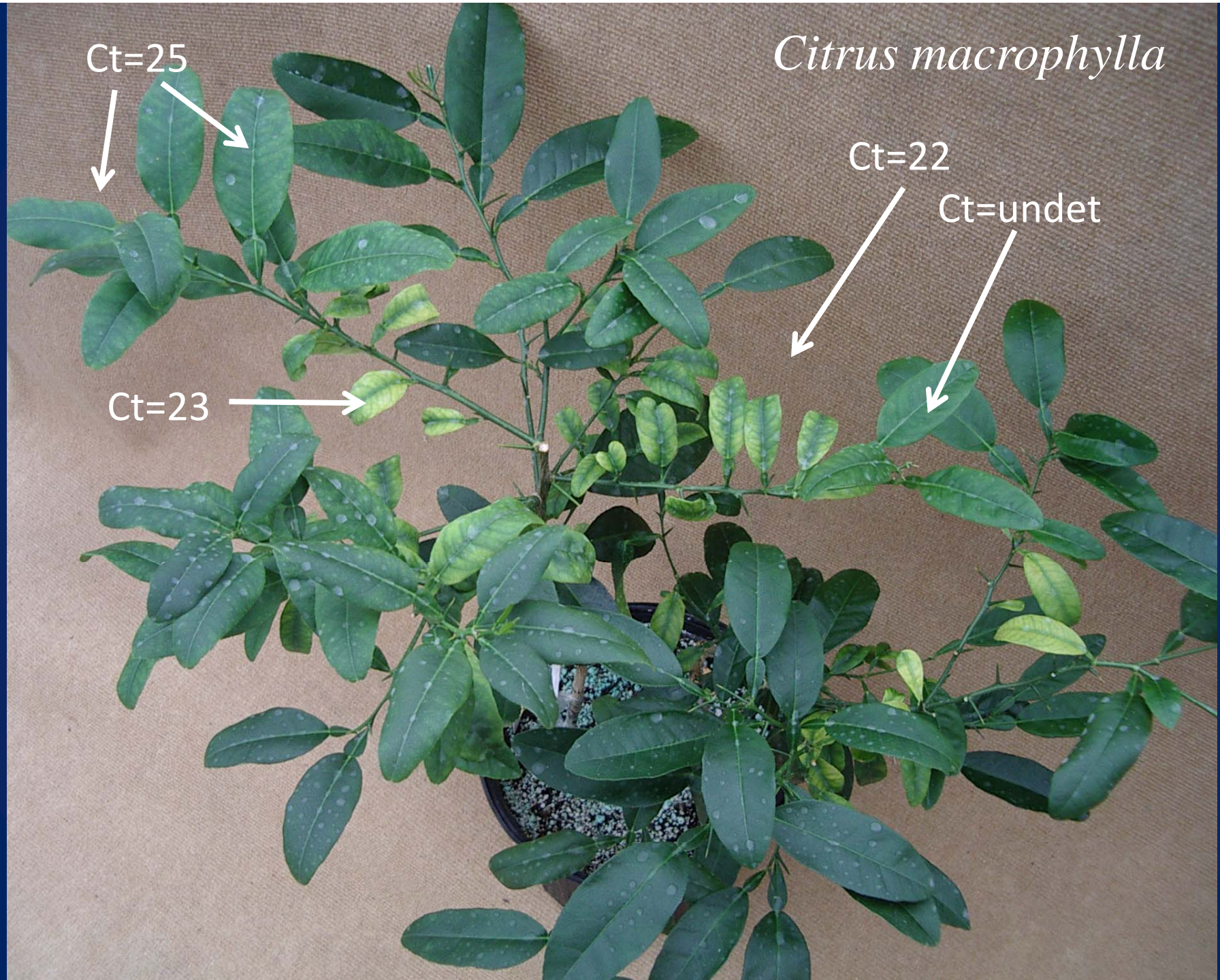
*Citrus macrophylla*

Ct=25

Ct=22

Ct=undet

Ct=23



## **Disease Symptoms Are Correlated with Abnormal Sugar Distribution**

Phloem blockage (phloem necrosis, callose deposits)



Disruption of sugar movement pathway –  
lack of sugar export



Starch granules accumulate and disrupt  
chloroplasts



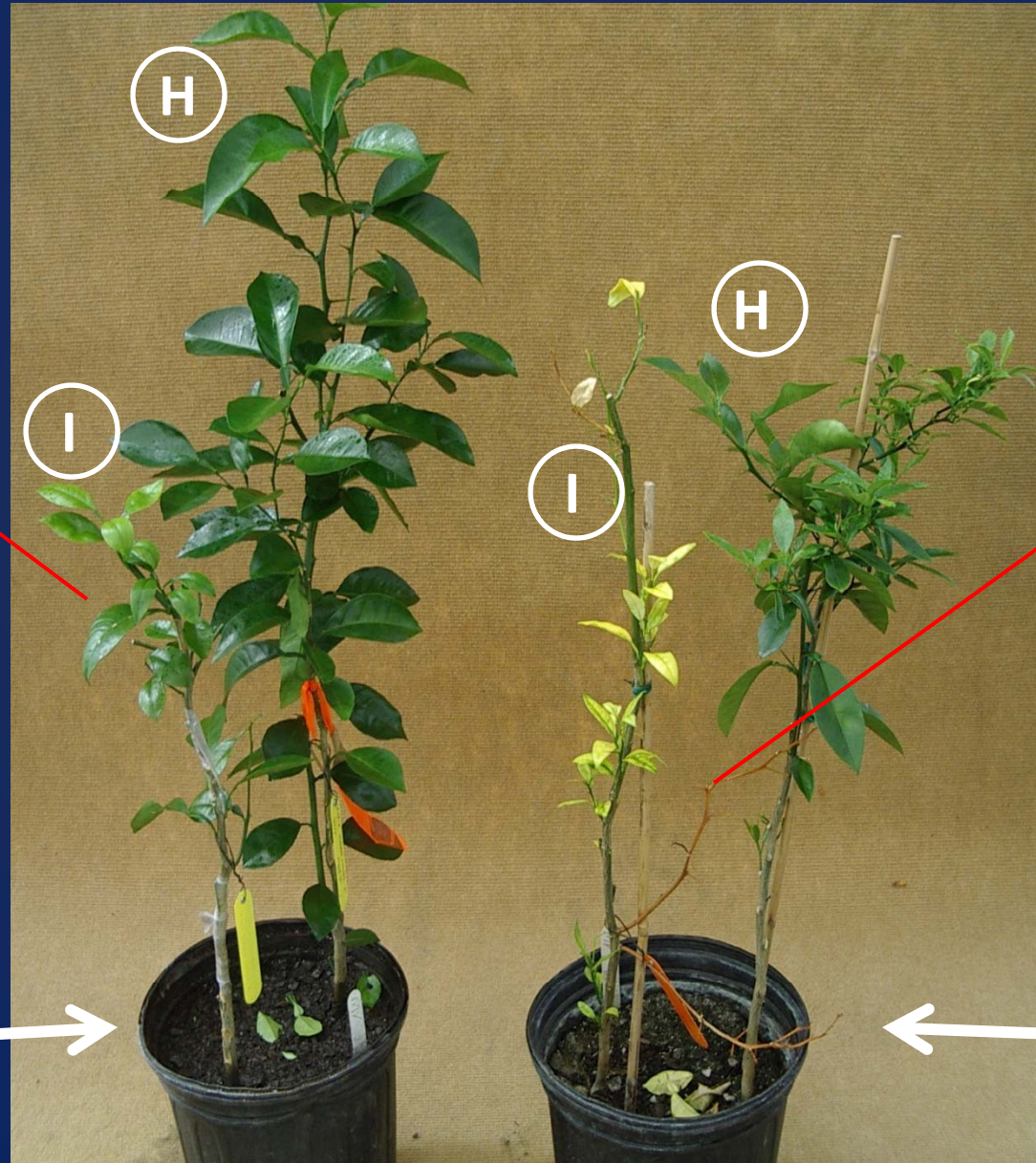
Chlorotic symptoms on leaves



# Effect of light on HLB symptoms development



Grown in the  
greenhouse



Grown under  
24h light



Severity of symptoms increases  
when plants are incubated under continuous light

Eureka lemon

Greenhouse conditions

24 hours light growth room





Symptoms in greenhouse



Sour  
orange

Symptoms under continuous light



Cleopatra  
mandarin





# Typical light room symptoms





# Non-inoculated sweet orange plants of 5 different cultivars



Hamlin

Pineapple

Madam Vinous

Rohde Red

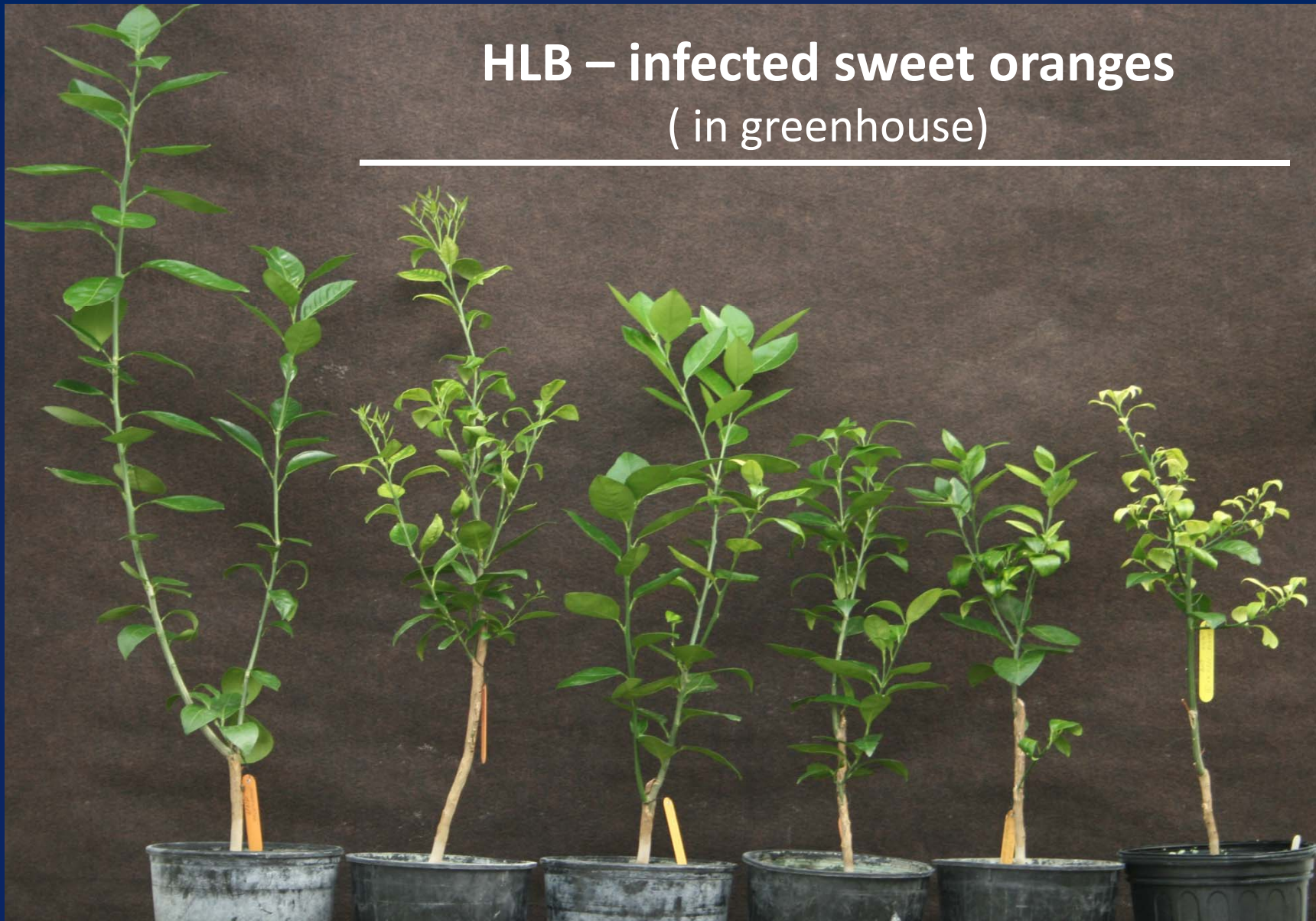
Valencia

Valencia

1-18-31



## HLB – infected sweet oranges ( in greenhouse )



**Healthy  
Hamlin**

**Hamlin**

**Rohde Red  
Valencia**

**Pineapple**

**Valencia  
1-18-31**

**Madam Vinous**



## Effect of HLB on grapefruit (in greenhouse)



Marsh grapefruit

Rio Red Grapefruit



Healthy plants



HLB-infected plants



Marsh Grapefruit

Rio Red Grapefruit

# Summary:

## 1. Susceptibility to Las:

Although Las was able to multiply in all tested citrus genotypes, there was a range of responses in different varieties from extreme tolerance to high sensitivity to HLB infection.

## 2. Distribution of Las within a tree:

In a particular plant higher titers are often associated with symptomatic areas.

## 3. Las titer and severity of disease:

When different citrus genotypes are compared, there is no clear correlation between bacterial titer and severity of disease.

## 4. Light intensity and duration greatly affect symptoms development.

# **Important questions:**

**What makes some citrus hosts tolerant and others – sensitive to HLB infection?**

**Can plants be modified to become resistant or tolerant?**