International Perspectives on HLB and ACP Research

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TEXAS A&M UNIVERSITY-KINGSVILLE CITRUS CENTER

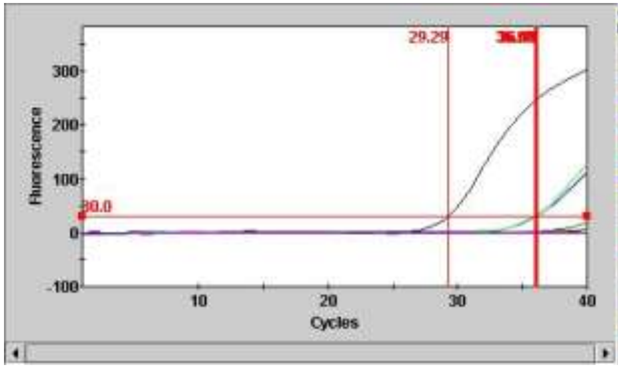
HLB/ACP – Complex Interactions

- Hosts – Citrus and other Rutaceae
- Pathogens–3 spp. of bacteria (+Phytoplasmas)
- Vectors – 2 species (+ 2 more?)
- Environments – weather, nutrition, soil, other pathogens

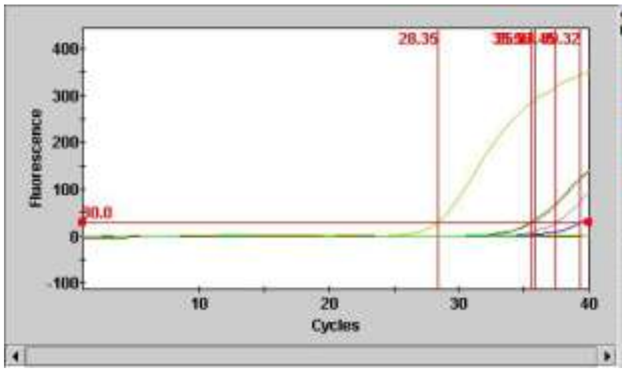
Research Overview

- Early Detection
- Antibiotics
- Psyllid research
- Interaction with Phytophthora
- Nutrition
- Resistance/Tolerance

Early Detection



Site ID	Protocol	Sample ID
[illegible]	PTBTMarch	77nextgrape
A2	PTBTMarch	77grape
A3	PTBTMarch	94
A4	PTBTMarch	95
A6	PTBTMarch	96
A7	PTBTMarch	97
[illegible]	PTBTMarch	98
A9	PTBTMarch	99
A10	PTBTMarch	101
A11	PTBTMarch	102
A12	PTBTMarch	103
[illegible]	PTBTMarch	LasPOS
A14	PTBTMarch	LamPOS
[illegible]	PTBTMarch	Healthy
[illegible]	PTBTMarch	NTC



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A6	PTBTMarch	LasPOS
A7	PTBTMarch	LamPOS
[illegible]	PTBTMarch	Healthy
A9	PTBTMarch	NTC

Conventional PCR with primers A2/J5. None of the samples that showed border line Ct showed amplicon.

Early detection of *Candidatus* Liberibacter asiaticus using root samples of 'Valencia' sweet orange on sour orange rootstock

Leaf sample:

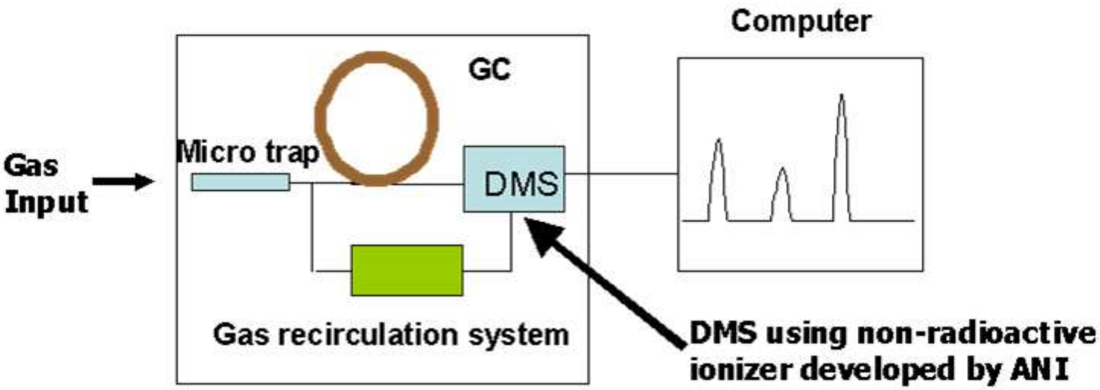
1. Leaf disease symptom – Depend on Environmental and Host factors
2. Uneven distribution in canopy and variation of symptomology
3. Expertise is needed to differentiate disease symptom from stress

Root sample:

1. Fibrous roots will serve as a better diagnostic sample compared to the leaves for early detection of Las in citrus trees – From trees without symptomatic leaves
2. Fibrous root samples collected near the tree trunk produced consistent and better detection of Las compared to the roots collected farther
3. In Texas, HLBfpr qPCR produced unreliable results with root sample
4. We suggest that roots are the primary place for Las colonization of the citrus plants regardless the fact that the psyllid inoculates the bacterium in the leaves
5. Most probably, Las moves to the roots through the vascular tissue where it finds a more stable environment to multiply and reach enough population to move to the canopy or part of the canopy when conditions are favorable. The same way if conditions in the canopy are not favorable, the roots may function as reservoir for the bacterium
6. More uniform Las distribution compared to canopy

VOC Sensor: EZKnowz™ hardware
(UC Davis/ EZDx)

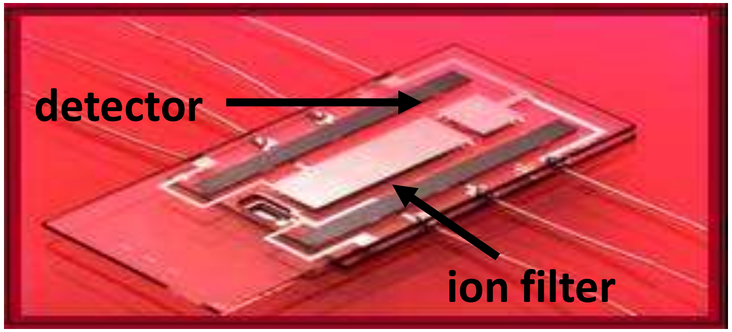
- EZKnowz™ combines:
  - Sampling system
  - Gas chromatography
  - Differential ion mobility
  - Add algorithm and data analysis on-board

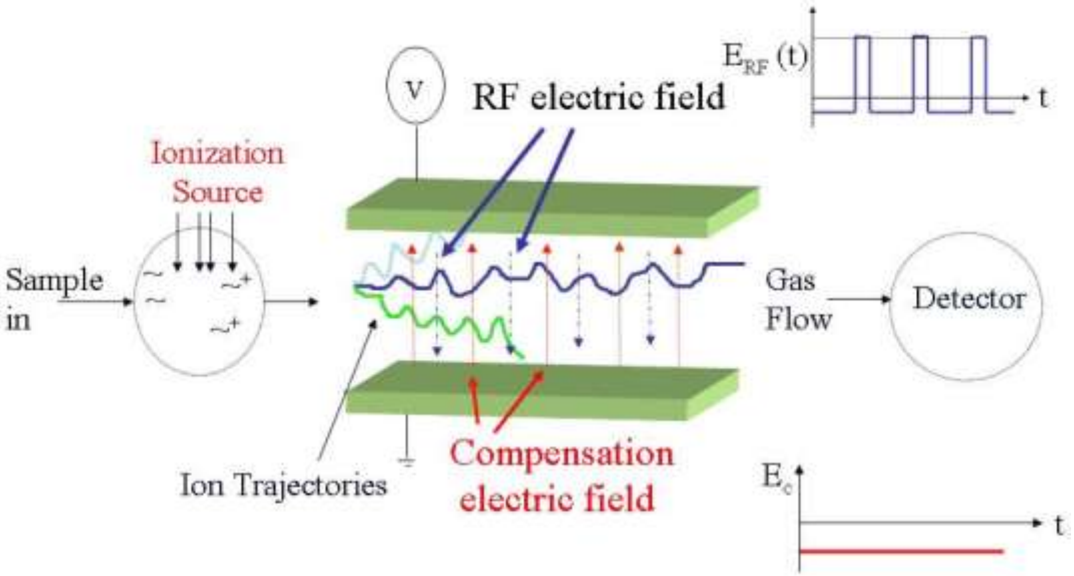


- Battery and/or solar powered.
- 10 minute now, goal 1 min

Differential mobility spectrometry (DMS)
(UC Davis/ EZDx)

- "Sniffer" sensitive to below ppb levels
- Robust and reagentless
- Software upgrades for new applications
- Compact and portable
- Low cost-of-ownership
- Minimal maintenance





Antibiotics?

- 1970s-1980s – South Africa; trunk injection with tetracycline hydrochloride or PMT.

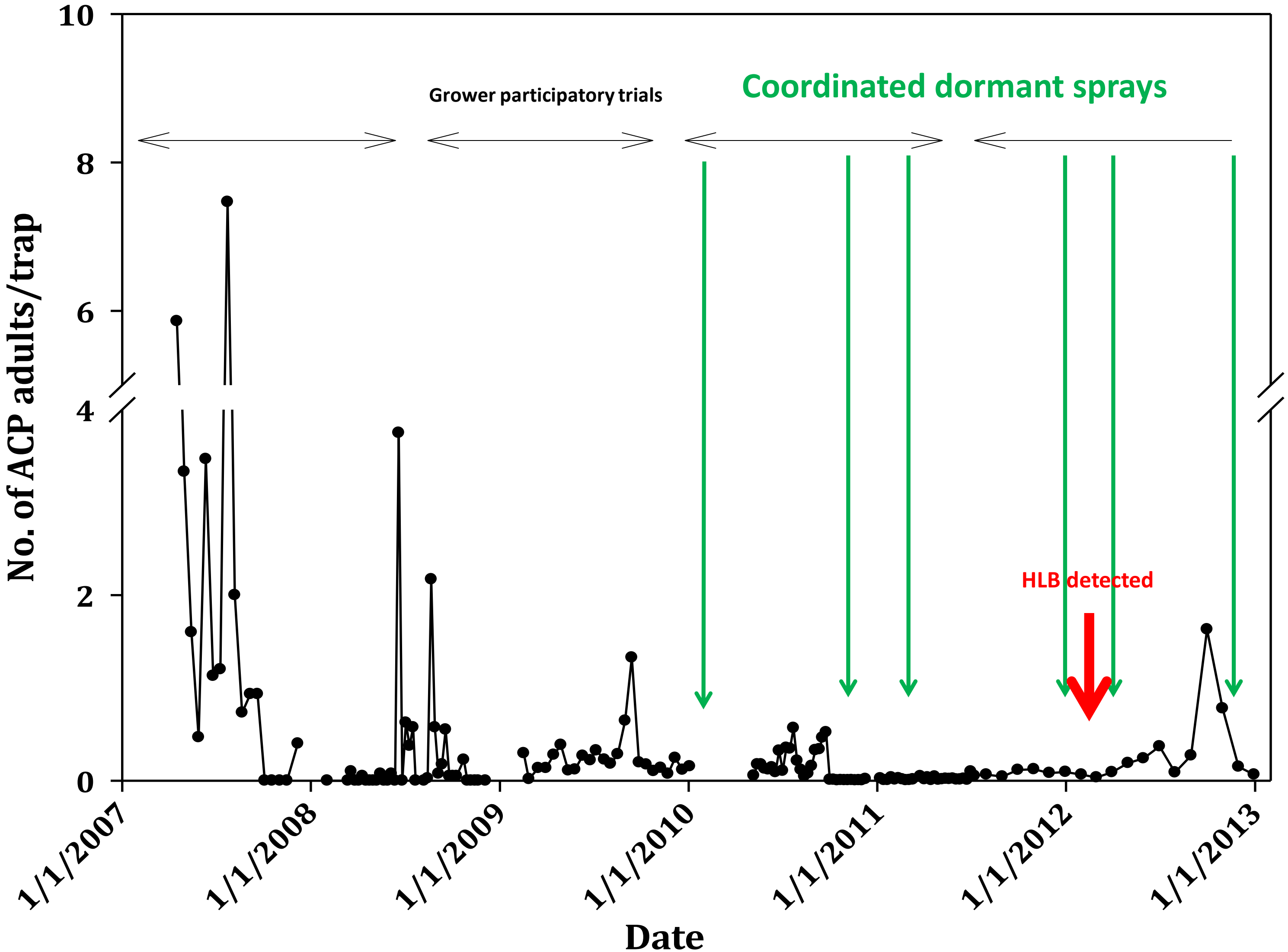
  Phytotoxicity; reduced but did not cure trees; labor intensive

- 2000s – Univ.Florida. Renewed interest with streptomycin. Soil and trunk applications in pot trials

Research on Psyllid control

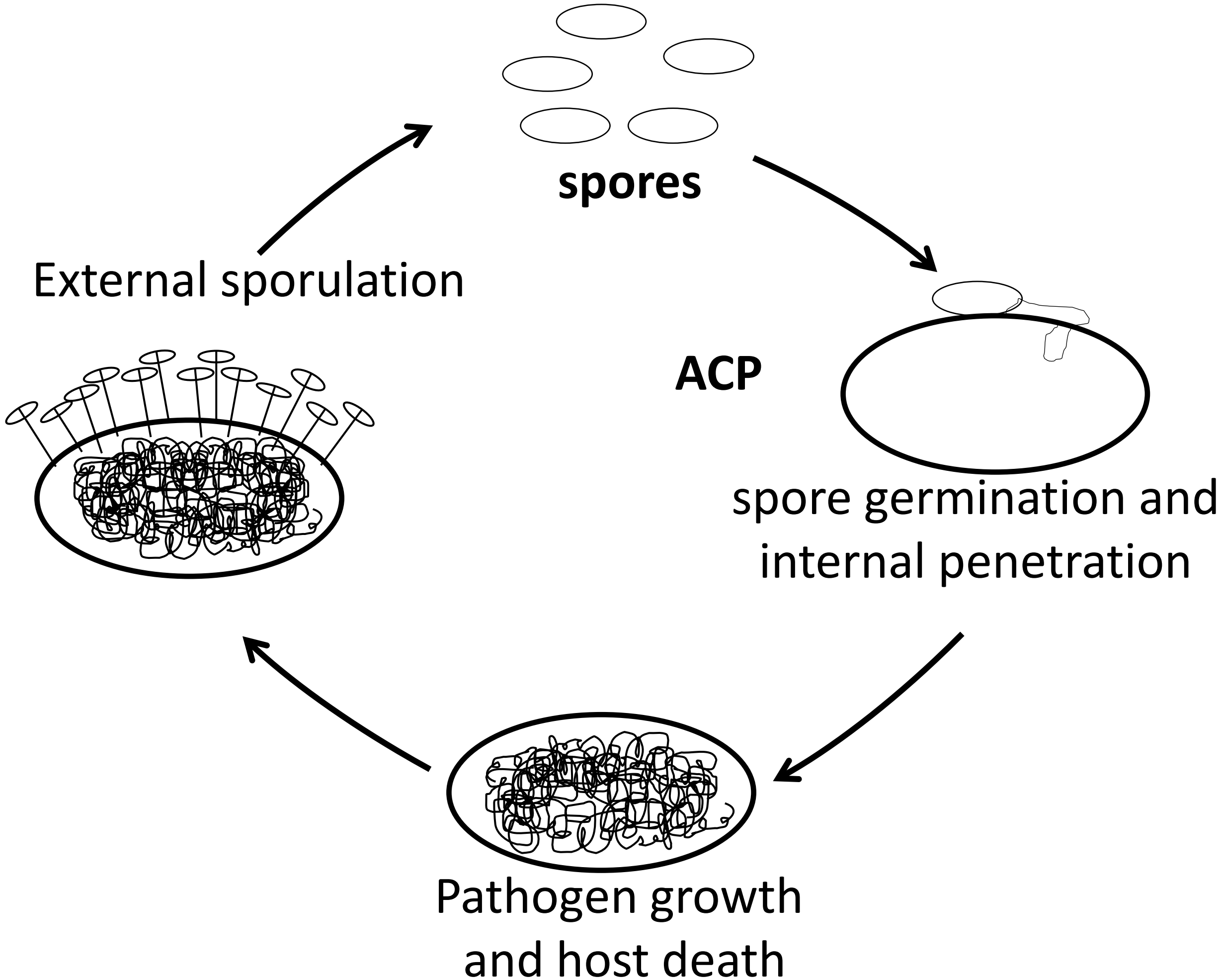
- AWM / Perimeter Sprays
- Biological control
- NuPsyllid project

Impact of AIMS on ACP Densities





Pathogenic fungus: *Isaria fumosorosea (Ifr)*



Kills psyllids within 4 days

Infected Asian citrus psyllids from trials

Tamarixia radiata

Modified mosquitoes for malaria control

NuPsyllid Project (Multi-lab; Multi-state)

- Develop, mass rear and release psyllid which are unable to transmit HLB
- Different approaches including:
  - Using viruses
  - Wolbachia
  - ScFv (fusion protein) transmission blocking

Psyllids, Las and Hosts

- Univ.Florida – psyllid ability to acquire Las is host dependent (70% acquisition from Rough lemon, 40% from lime and orange; <5% from grapefruit)

(Stelinski lab, Lake Alfred – Citrus Industry magazine)

Phytophthora and HLB

- Dr Jim Graham (UFL):
- Stress tolerance in HLB affected trees significantly reduced by Phytophthora infection of roots (loss of fibrous roots). Trees with HLB had up to 40% less fibrous roots than HLB negative trees

Maury Boyd

Photo: Julian Sauls

Maury Boyd (L); Neighbor (R)(J.Sauls)

The influence of the rootstock on greening fruit symptoms

S P VAN VUUREN and J N MOLL *Citrus and Subtropical Fruit Research Institute, Nelspruit*

Key Words: Greening, rootstocks

Abstract

The percentage greening fruit symptoms were assessed in orchards with different scion and rootstock combinations in the same block.

No difference occurred where two scion selections were used on the same rootstock.

Significant differences were found where different rootstock cultivars were used with the same scion. The highest percentage greening occurred where *Poncirus trifoliata* was used as a rootstock. The reason for the higher greening infection is still unexplained, but it is possible that it has an influence on the flush rhythm of a tree. *P. trifoliata* has a dwarfing effect on scions and, because of this characteristic, may extend the flushing period. The greening vector, breeding and feeding on young growth, will have an extended period of feeding on such trees and therefore transmit the greening agent more frequently.

No differences occurred where four *P. trifoliata* selections were compared as rootstocks.

Uittreksel

DIE INVLOED VAN DIE ONDERSTAM OP VERGROENING VRUGSIMPTOME

Die persentasie vergroening vrugsimptome is bepaal in boorde waar verskillende bo- en onderstamme gebruik is in dieselfde blok.

Geen verskille het voorgekom waar twee bo-stam seleksies op dieselfde onderstam gebruik is nie. Betekenisvolle verskille het voorgekom waar 'n bo-stam seleksie op verskillende onderstam-cultivars gebruik is. Die hoogste persentasie vergroening het voorgekom waar *Poncirus trifoliata* as onderstam gebruik is. Die rede vir die hoër vergroening besmetting is nog onverklaarbaar maar dit is moontlik dat die onderstam 'n invloed het op die ontwikkeling van nuwe groei. *P. trifoliata* het 'n verdwergende invloed op die bostam en, as gevolg van die eienskap, mag dit die periode waar nuwe groei ontwikkel verleng. Die vergroeningsvektor, wat op jong groei voed en teel, sal dus 'n verlengde periode hê om op sulke bome te voed en daarom die vergroeningsorganisme meer dikwels oordra.

Geen verskille het voorgekom waar vier *P. trifoliata* seleksies as onderstam vergelyk is nie.

Introduction

McClean and Schwarz (1970) found that all commercial citrus cultivars in South Africa are susceptible to greening although certain cultivars and selections are more tolerant. They also found that rootstock cultivars such as rough lemons, *Troyer citrange* and *Poncirus trifoliata* are susceptible.

Cheema, *et. al.* (1982) compared the susceptibility of various rough lemon selections and other rootstock cultivars to greening disease in the Philippines. While five rough lemon selections were found to be tolerant none of the other rootstock cultivars were.

Gonzales, *et. al.* (1972) reported on the resistance or tolerance of 110 citrus cultivars to greening disease and mentioned that greening is not considered to be a rootstock-scion disease. Marloth (1950) did not find differences in the occurrence of greening in sweet orange on rough lemon and sweet orange rootstocks. This paper provides evidence that the rootstock cultivar influences the occurrence of greening fruit symptoms in the scion.

Procedure

A. Comparison of two scion selections

Twenty trees each of 16-year old Naude Valencia and Olinda Valencia were used. Both scion selections were nucellar sources and were planted alternatively in the same orchard on Empress mandarin rootstock. All the fruit at harvest were grouped in the categories of marketable and greened fruit according to external fruit symptoms.

Table 1. The occurrence of greening fruit symptoms or two sweet orange selections on the same rootstock
Tabel 1. Die voorkoms van vergroening vrugsimptome op twee soetlemoen seleksies op dieselfde onderstam

Scion/Bostam	Rootstock/Onderstam	% Greening/% Vergroening
Naude Valencia	Empress mandarin/mandaryn	44,2*
Olinda Valencia	Empress mandarin/mandaryn	36,9

**Differences not statistically significant*
Verskille nie statisties betekenisvol nie

Table 2. Greening fruit-symptoms in a sweet orange selection with different citrus cultivars as rootstocks
Tabel 2. Vergroening vrug-simptome op 'n soetlemoen seleksie met verskillende sitrus cultivars as onderstamme

Rootstock/Onderstam	Scion/Bostam	% Greening/% Vergroening
Orchard 1/Boord 1		
Empress mandarin/mandaryn	Naude Valencia	44,2 a*
Troyer citrange	Naude Valencia	49,8 a
P. trifoliata	Naude Valencia	81,8 b
Orchard 2/Boord 2		
Empress mandarin/mandaryn	Naude Valencia	9,5 a
P. trifoliata	Naude Valencia	41,1 b

**Percentages followed by the same letter do not differ statistically.*
Persentasies gevolg met dieselfde letter verskil nie statisties van mekaar nie.

Table 3. Percentage greening fruit symptoms in sweet orange with different *Poncirus trifoliata* selections as rootstocks
Tabel 3. Die persentasie vergroening in vrugte van soetlemoen op verskillende *Poncirus trifoliata* seleksies as onderstamme

P. trifoliata selection/seleksie	Scion/Bostam	% Greening/% Vergroening
English	Naude Valencia	41,4 a*
Kryder	Naude Valencia	50,3 a
Christian	Olinda Valencia	46,3 a
Benecke	Olinda Valencia	59,7 a

**Differences not statistically significant.*
Verskille nie statisties betekenisvol nie.

continued on page 10

Florida Rootstock Findings

- Some rootstocks appear to allow trees to remain productive after infection:
- Simple and complex hybrids (USDA)
- Complex hybrids (UF)

HLB tolerance from new rootstocks?

Vernia/Orange #4 Vernia/Orange #19

New photos of trees PCR+ since last September – St. Helena

Photo from Dr Jude Grosser, UFL

68-1G-26-F2-P12	10	0	2	20%
68-1G-26-F4-P2	12	1	2	17%
68-1G-26-F4-P6	***13***	***0***	***0***	***0%***
68-1G-26-F6-P20	17	0	3	18%
69-LTX-AM-F14 P37	4	0	2	50%
Aqua 1803	19	3	5	26%
CLEO	**16**	**0**	**4**	**25%**
FG 1702*	***2***	***0***	***0***	***0%***
FG 1707	3	0	1	33%
FG 1709	***4***	***0***	***0***	***0%***
FG 1712	1	1	1	100%
FG 1714	1	1	1	100%
FG 1715*	***1***	***0***	***0***	***0%***
FG 1722	1	0	1	100%
FG 1731	5	0	1	20%
FG 1733	5	1	1	20%
FG 1792*	***2***	***0***	***0***	***0%***
FG 1793	5	1	2	40%
FG 1794*	***2***	***0***	***0***	***0%***
Kuharske	**63**	**24**	**58**	**92%**
MG11	40	1	5	13%
Orange 1804	18	3	7	39%
Pink 1802	18	3	4	22%
Rough Lemon	**18**	**3**	**10**	**56%**
Swingle	**20**	**6**	**14**	**70%**
Volk	**20**	**7**	**17**	**85%**
White 1801	11	1	5	45%
White 1805	19	1	4	21%
Yellow 1800	11	1	2	18%
Total	305	58	152	50%

USDA ARS FL Rootstocks

- Sweet orange on 15 rootstocks infected with HLB:
- No resistance , but some tolerance in trees on US-897, US-802.
- US-897 seedlings symptomless (PCR +).

Chimeras on Valencias
South Africa (SP van Vuuren)

South African Valencias 2010

S.P.van Vuuren

Greening resistant (?) Valencia – 2013

S.P.van Vuuren

Trioza leaf damage

Greening inoculation test on 'Rio Red' Grapefruit

Non-Transgenic Buds grafted on infected rootstock

SD Transgenic Buds grafted on infected rootstock (Photo-E.Mirkov)

Transgenic Varieties with Spinach Defensins (SoD2, SoD7—Gen 2-4 Plants)

Varieties

‘Frost Eureka’ Lemon
‘Frost Lisbon’ Lemon
‘Limoneria 8A’ Lemon

‘Rio Red’ Grapefruit

‘Ruby Red’ Grapefruit

‘Hamlin’ Sweet Orange

‘Marrs’ Sweet Orange

‘Rhode Red’ Valencia

Rootstocks

‘Carrizo’

‘C22’

‘Flying Dragon’

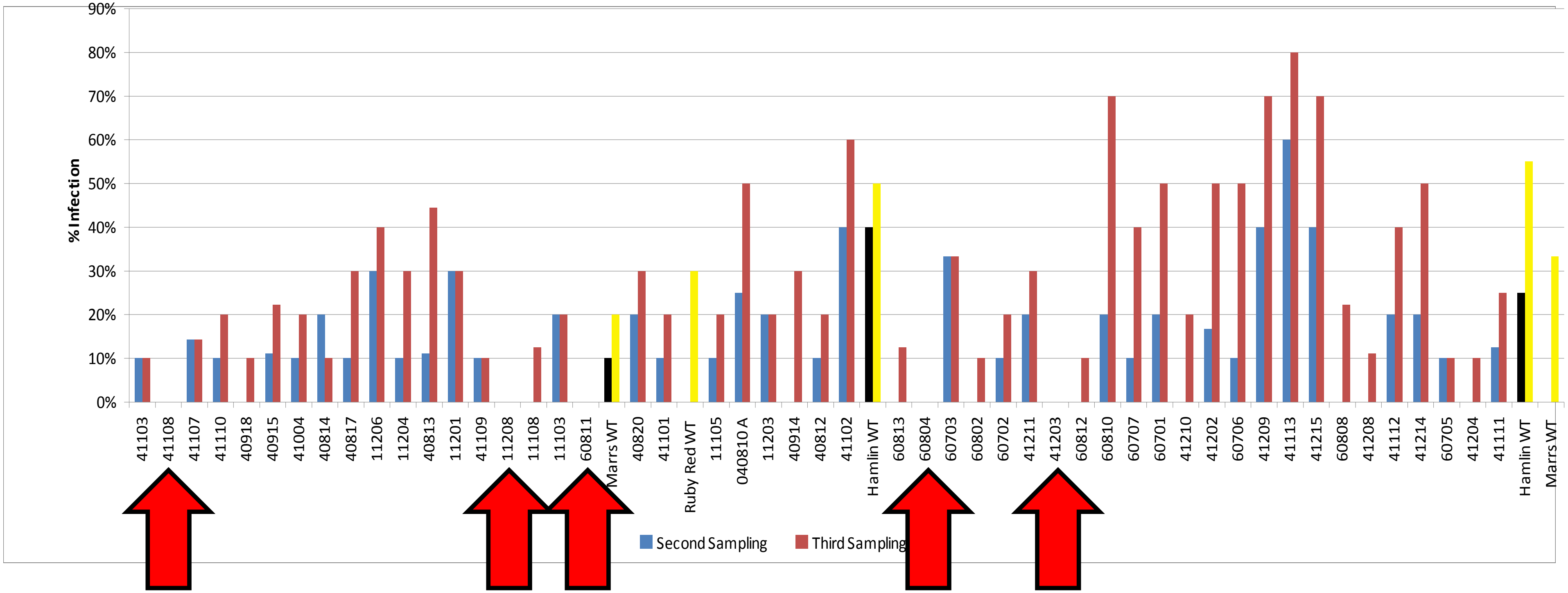
‘Swingle’

Psyllid House Screening: Generation 2 , 3 and 4
SoD2 or/and SoD7, with or w/o Signal Peptide –
(E.Mirkov)

Psyllid House Challenges of Transgenic Citrus SoD2 & SoD7 Lines

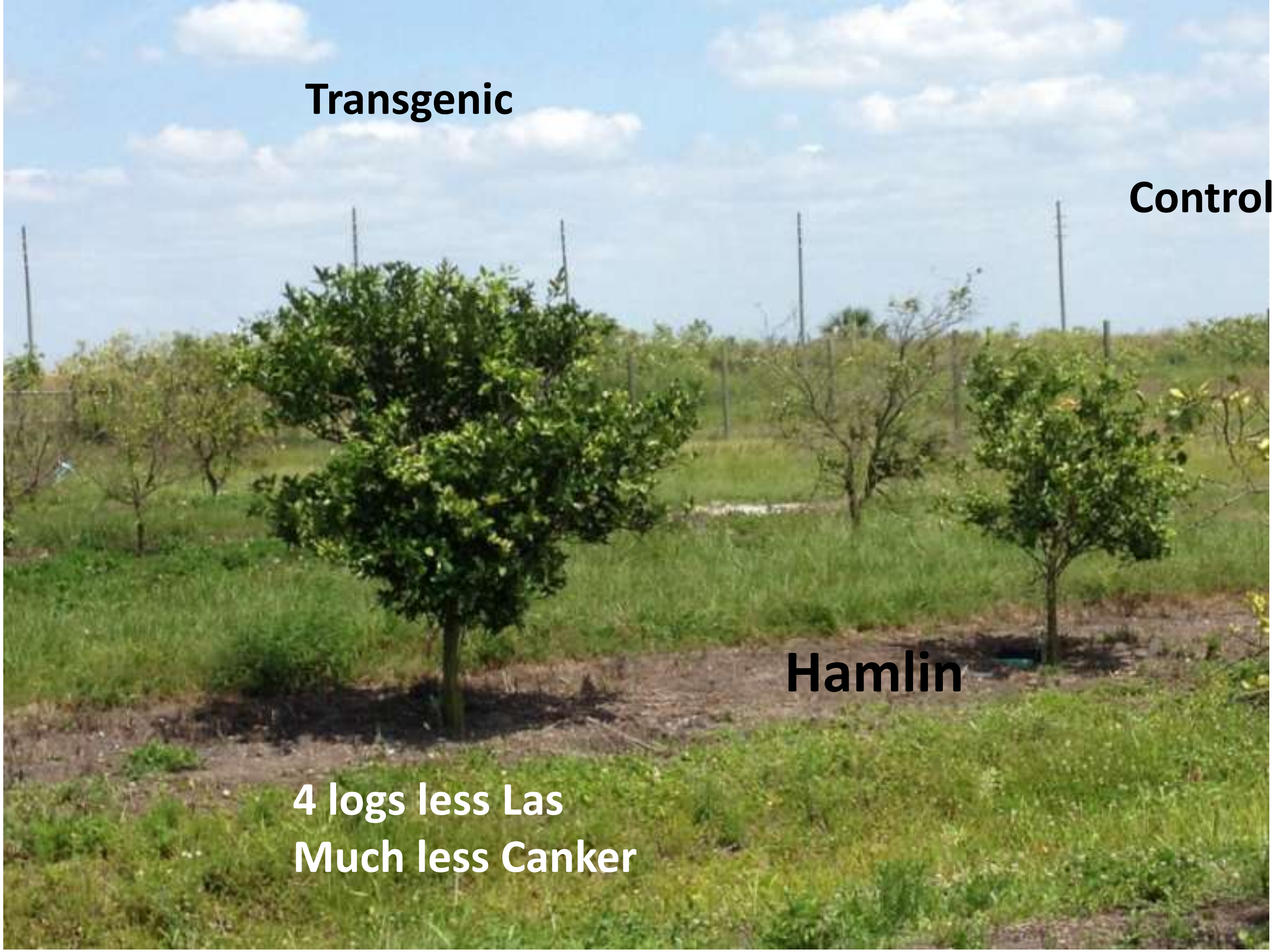
Disease Immunity

Generation 3
~1 Year in Psyllid House



Fourth Sampling; 5 months later—Still have 5 immune lines!

Photo- E.Mirkov



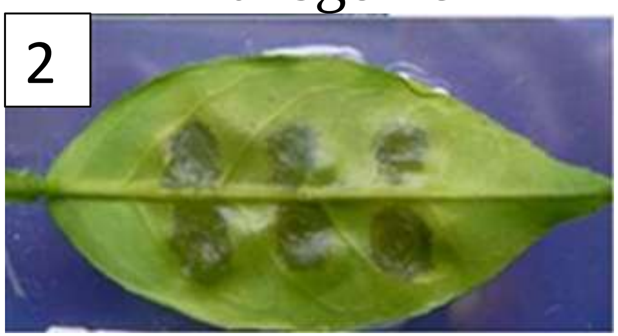
Grapefruit Plants Challenged with Pathogens – E.Louzada

Citrus Canker

Xanthomonas axonopodis pv. *citri*

Non-Transgenic

Transgenic



Phytophthora

P. nicotianae

Non-Transgenic

Transgenic

Regulatory – EPA+USDA+FDA

Study	Cost ($1000's)
Recombinant protein production	300
Antibody production	100
ELISA method development	100
ELISA Validation	125
Western method development	50
Composition/Expression/ Agronomcs	500
Southern blot	100
Sequencing	125
Within generation analysis	100
Efficacy	200
Inheritance	50
Acute oral mouse	100
Aa homology search	50
Toxin homology search	50

Study	Cost ($1000's)
Thermolability + in-vitro digestibility	100
42-day broiler	150
90-day rat feeding (full tox profile)	275
90-day rat feeding - China (full tox profile)	75
Human health risk assessment	50
Protein equivalency – recombinant v. plant-made	75
Non-target organisms and ecotoxicology	200
Honeybee toxicity	30
Non-target risk assessment	50
Product characterization (gene description, transformation, etc.)	25
Event PCR method	200
Certified ref. materials (EU)	115
Total	3295

Concluding Remarks

- Some progress on earlier detection
- Better sustainable ACP management for short-medium term.
- NuPsyllid ? (medium-long term)
- Management of tree health (Phytophthora, nutrition)(immediate)
- Resistance/ Tolerance must be long term goals (natural or transgenic)