Outcome Group 3: Topic Driven Discussions – keep citrus trees free of ACP and HLB

Topic 3: Host response studies for discovery of pre-symptomatic HLB biomarkers and characterization of HLB tolerance/resistance.

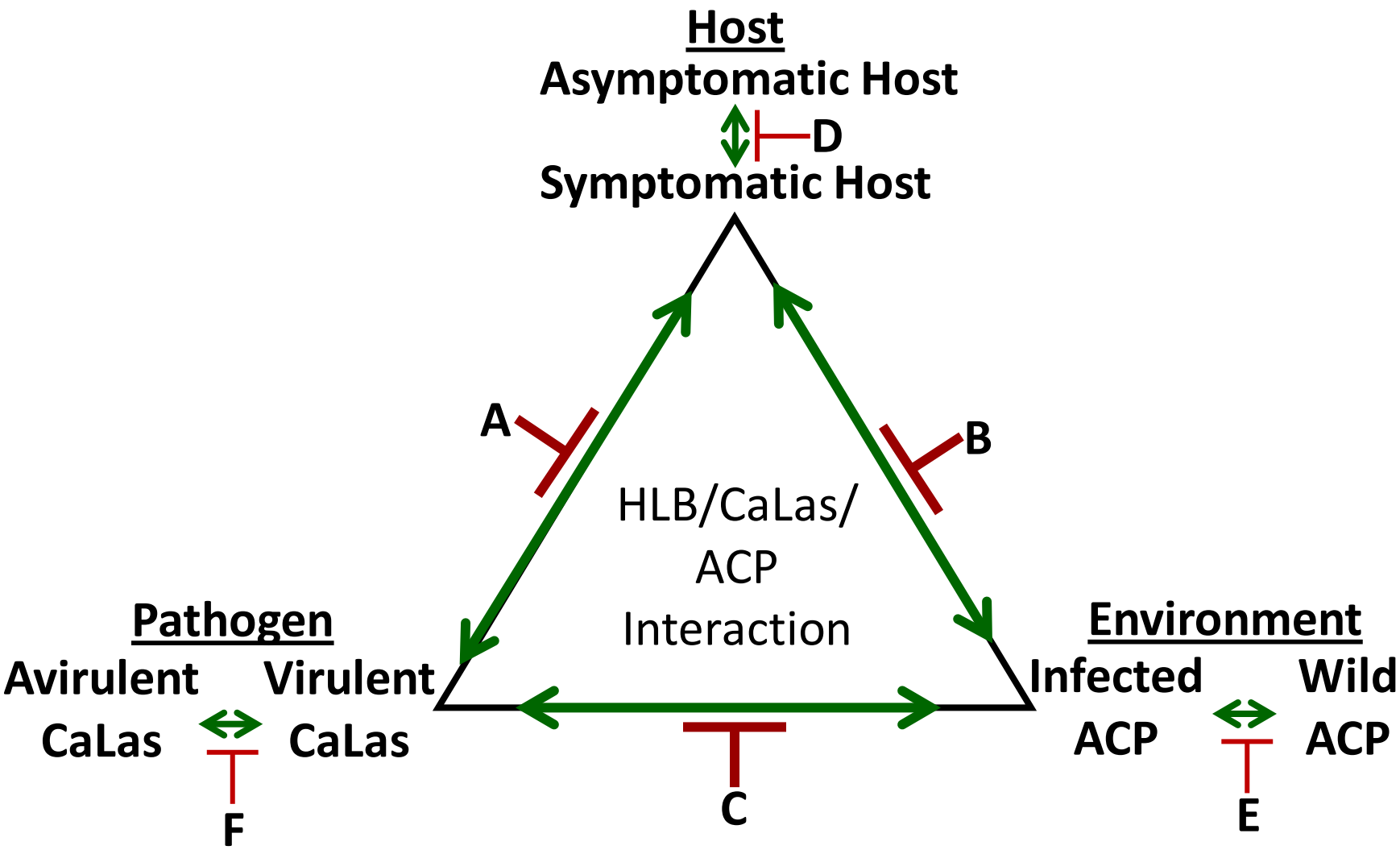
3rd Annual Citrus Health Research Forum
August 27-30, 2012; Ft. Collins, CO

Abhaya M. Dandekar
Plant Science Department
UC DAVIS
University of California, Davis

Relevance?

- Early detection and early therapy are key to decreasing morbidity of a number of cancers in addition to and as an alternative to surgery
  - Understanding the biology of the disease
  - Defining pathways and identifying early molecular biomarkers of disease
  - Developing and validating treatments
  - Identifying resistance to therapy early
- Early detection and therapy are key to the AIDS epidemic
  - Early detection of HIV via antibodies
  - Early treatment with HAART
  - Monitoring treatment via CD4

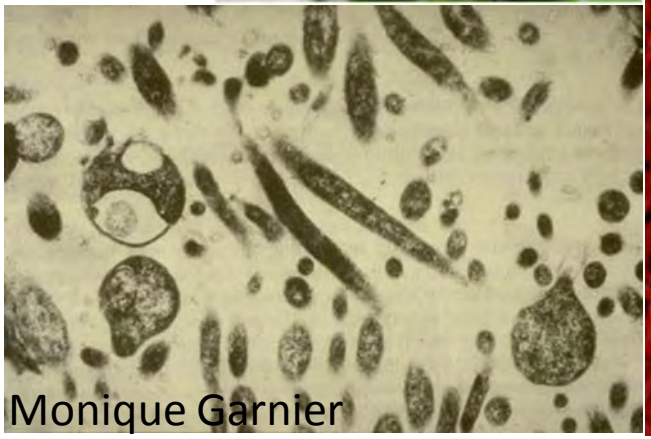
Early host responses are key to defining the biology of HLB disease



OG3: “keep citrus trees free of ACP and HLB” – A, B & D

What is the Genetic and/or Biochemical basis of the Phenotypic Host Response to HLB?

- Characteristic/Unique Phenotypic response in leaf and fruit tissues
- Variation of bacterial titer
- Variation of phenotypic response among and within different citrus genotypes



4 Categories of Host Response Phenotypes based on Citrus Genotype

- Host responses of 30 genotypes examined
- Sensitive and Tolerant genotypes
- Lack of correlation of bacterial titer and phenotype or Genotype

TABLE 1. Response of different *Citrus* genotypes to grafting with buds from '*Candidatus* Liberibacter asiaticus'-infected trees

Citrus or relative	'*Ca.* L. asiaticus'[a]	Symptom description
Citrus halimii B. C. Stone	++	Sensitive—chlorosis, vein corking, death
Nules clementine (*C. reticulata* Blanco)	++	Sensitive—chlorosis to tip leaves, reduced growth, death
Minneola tangelo (*C.* × *tangelo* J. Ingram & H. E. Moore)	++	Sensitive—chlorosis to tip leaves, reduced growth
Valencia sweet orange (*C. sinensis* (L.) Osbeck)	+++	Sensitive—chlorosis including tip, reduced growth, death
Madam Vinous sweet orange (*C. sinensis* (L.) Osbeck)	+++	Sensitive—chlorosis including tip, reduced growth, death
Duncan grapefruit (*C. paradisi* MacFadyen)	++	Sensitive—chlorosis including tip, reduced growth, death
Ruby red grapefruit (*C. paradisi* MacFadyen)	++	Sensitive—chlorosis to tip leaves, reduced growth
Siamese Sweet pummelo (*C. maxima* (Burm.) Merr.)	++	Variable—mild symptoms in GH, strong under 24 h of light
Ling Ping Yau pummelo (*C. maxima* (Burm.) Merr.)	++	Variable—mild symptoms in GH, strong under 24 h of light
Hirado Buntan Pink pummelo (*C. maxima* (Burm.) Merr.)	++	Variable—strong symptoms in young and old leaves
C. amblycarpa (Hassk.) Ochse	+++	Variable—chlorosis and some reduction of growth
Cleopatra mandarin (*C. reticulata* Blanco)	+++	Variable—chlorotic leaves, some reduction of growth
C. indica Tanaka	++	Variable—early chlorosis, severe under 24 h of light
Meiwa kumquat (*Fortunella crassifolia* Swingle 'Meiwa')	++	Variable—chlorotic leaves, plant growth reduced
Sun Chu Sha mandarin(*C. reticulata* Blanco)	+++	Moderately tolerant—scattered chlorotic groups of leaves
Sour orange (*C. aurantium* L.)	+++	Moderately tolerant—chlorosis under high light only
Volkamer lemon (*C. limonia* Osbeck 'Volkameriana')	+++	Moderately tolerant—scattered chlorotic groups of leaves
C. macrophylla Wester	+++	Moderately tolerant—scattered chlorotic groups of leaves
Swingle citrumelo (X *Citroncirus webberi* J. Ingram & H. E. Moore)	+++	Moderately tolerant—some yellowing of older leaves
Citron (*Citrus medica* L.)	++	Moderately tolerant—strong symptoms on older leaves
Palestine Sweet lime (*C. aurantifolia* (Christm.) Swingle)	+++	Moderately tolerant—some chlorosis on older leaves
Mexican lime(*C. aurantifolia* (Christm.) Swingle)	+++	Moderately tolerant—scattered chlorotic groups of leaves
Calamondin (X *Citrofortunella microcarpa* (Bunge) Wijnands)	+++	Moderately tolerant—mild symptoms on older leaves
Citrus micrantha Wester	+++	Moderately tolerant—mild symptoms on older leaves
Eureka lemon (*C. limonia* Osbeck)	++	Tolerant—no symptoms, chlorosis under 24 h of light only
Persian lime (*C. aurantifolia* (Christm.) Swingle)	+++	Tolerant—little or no symptoms
Carrizo citrange (X *Citroncirus webberi* J. Ingram & H. E. Moore)	+	Tolerant—little or no chlorosis
Severinia buxifolia (Poiret) Ten.	+	Tolerant—no distinct symptoms
Poncirus trifoliata (L.) Raf.	+/–	Inconsistent
Citrus latipes (Swingle)	+/–	Inconsistent

[a] Relative bacterial titers based on polymerase chain reaction analyses with '*Ca.* L. asiaticus'-specific primers sets, with +++ being highest and + usually being low but definitely positive. GH = greenhouse.

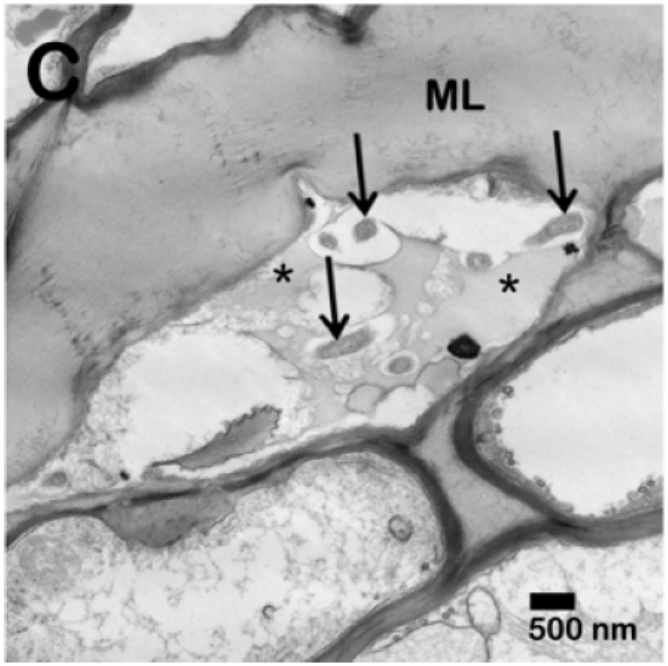
Valencia Sweet Orange

Eureka Lemon

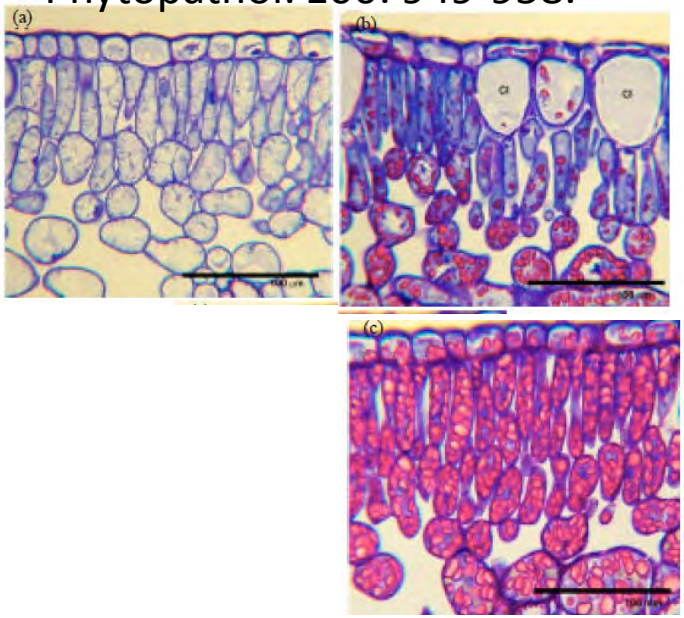
Folimonova et al., 2009. Phytopathol. 99:1346-1354.

Sequence of Host Response Events:

- Phloem plugging with callose and PP2 (CF831073) deposition
- Swelling of middle lemella between cell walls surrounding sieve elements
- Presymptomatic tissues (young flushes) contain large amounts of bacteria in phloem companion cells
- Phloem necrosis, companion and sieve element cell wall swelling
- Collapse of some phloem elements
- Back-up of sugar
- Starch accumulation
- Starch build-up in chloroplast with disruption of thylakoid membranes

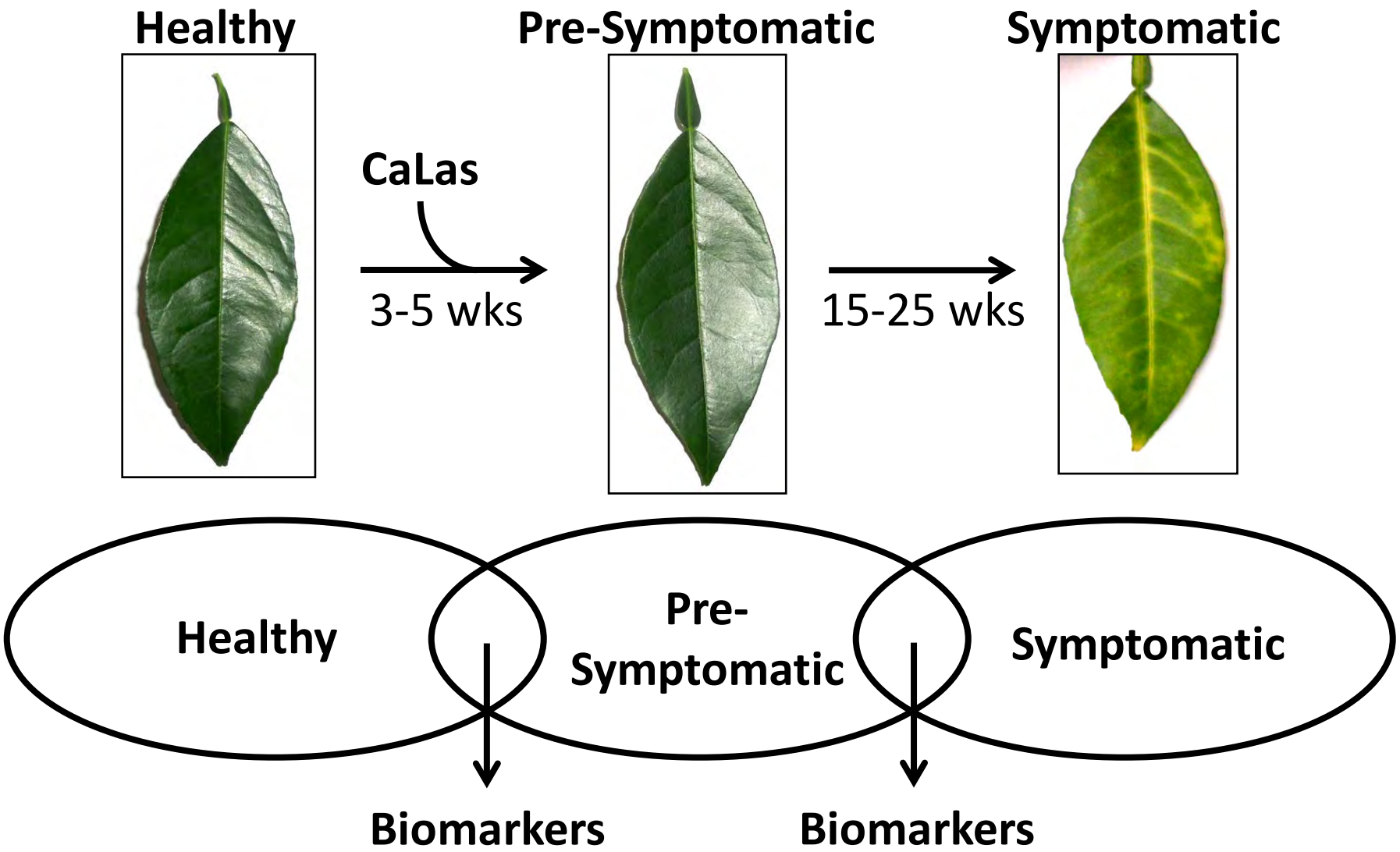


Folimonova and Achor. 2010 Phytopathol. 100: 949-958.



Achor et al., 2010. Plant Pathol J. 9: 56-64

Typical Longitudinal analysis of host responses by graft inoculation



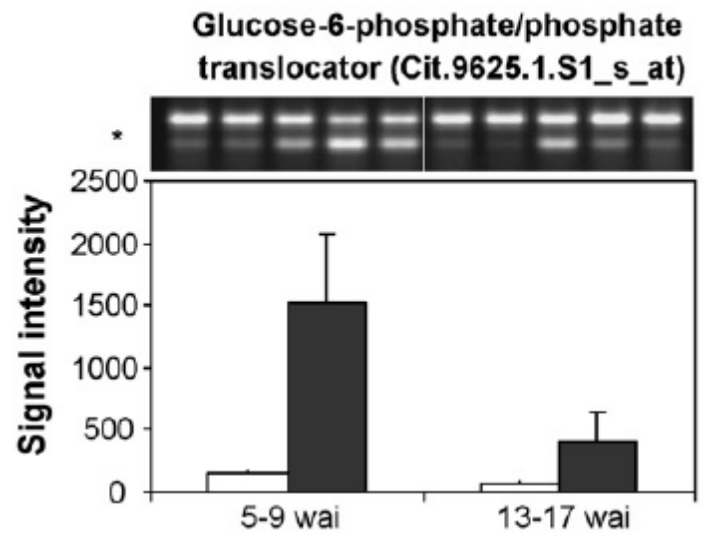
Are there specific host responses that indicate the early (pre-symptomatic) stages of CaLas Infection?

- Early descriptors (biomarkers) of CaLas infection before the appearance of visual symptoms
  - Transcriptome
    - Specific mRNA
    - Specific sRNA
  - Proteome
    - Specific proteins
    - Antibodies to specific proteins
  - Metabolome
    - Extractable metabolites
    - Volatile metabolites
- Genetic descriptor's of tolerance/resistance to CaLas
  - Transcriptome
  - Proteome
  - Metabolome

Transcriptome analysis using Microarrays

Number of genes differentially expressed in citrus leaves infected with *Ca.* L. asiaticus compared with non-infected leaves

	$P \leq 0.05$	$P \leq 0.01$	FDR ≤ 0.05	FDR ≤ 0.01
5–9 Weeks after inoculation				
Up-regulated	793	393	195	137
Down-regulated	218	153	84	50
Total	1011	546	279	187
13–17 Weeks after inoculation				
Up-regulated	1028	526	310	251
Down-regulated	668	409	205	136
Total	1696	935	515	387



- Longitudinal study of leaves
- Affimetrix citrus microarrays with 33,000 probe sets
- Expression levels of many genes are altered – Cell defense, transport, cellular organization, photosynthesis and carbohydrate metabolism
- Sucrose levels higher in infected leaves

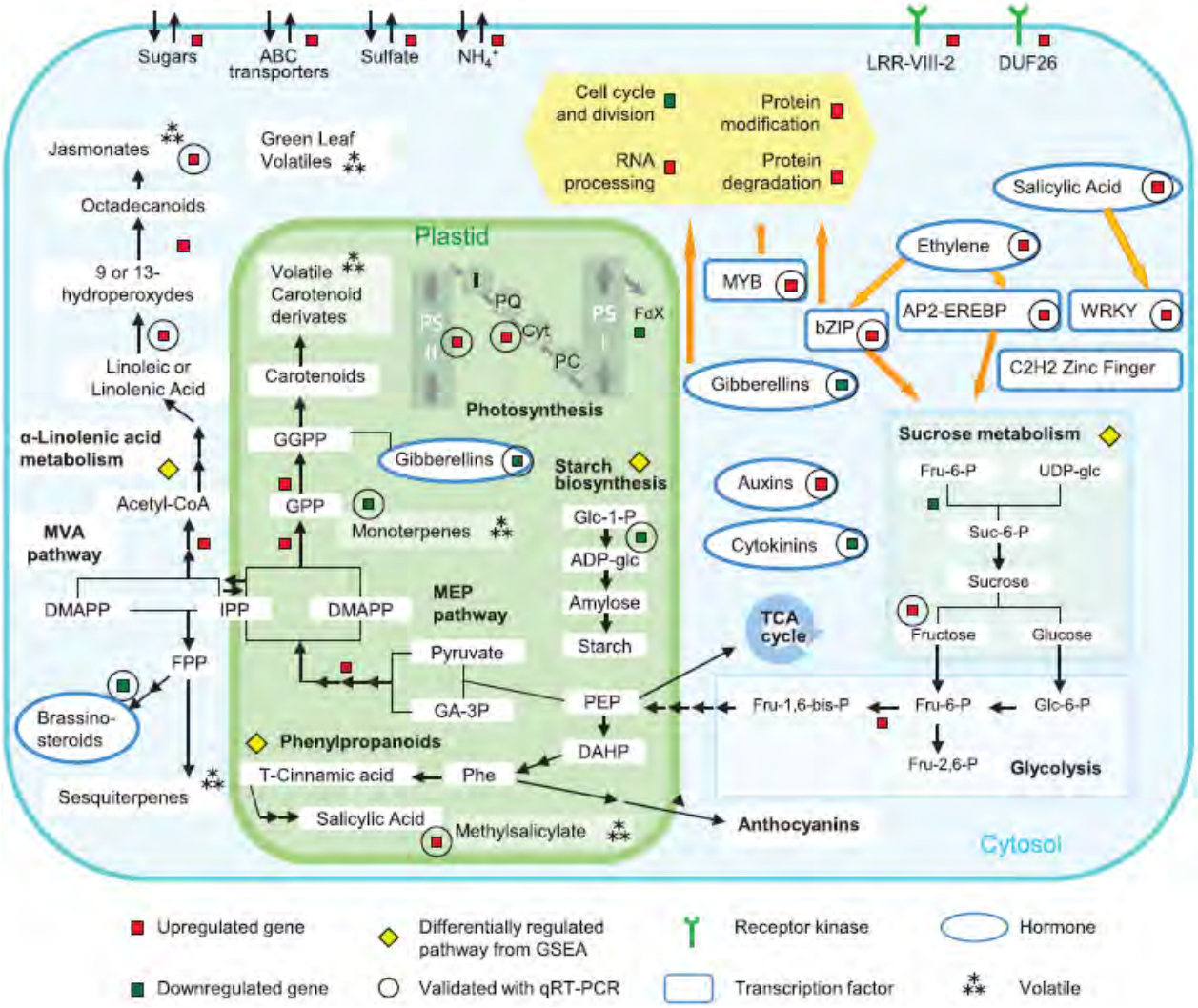
Albrecht & Bowman 2008. Plant Sci. 175: 291–306

pp2 gene up-regulation related to callose deposition

Kim et al., 2009Phytopathol. 99: 50-57.

Deep sequencing analysis (RNA-seq) of transcriptome of citrus fruit from asymptomatic and symptomatic trees

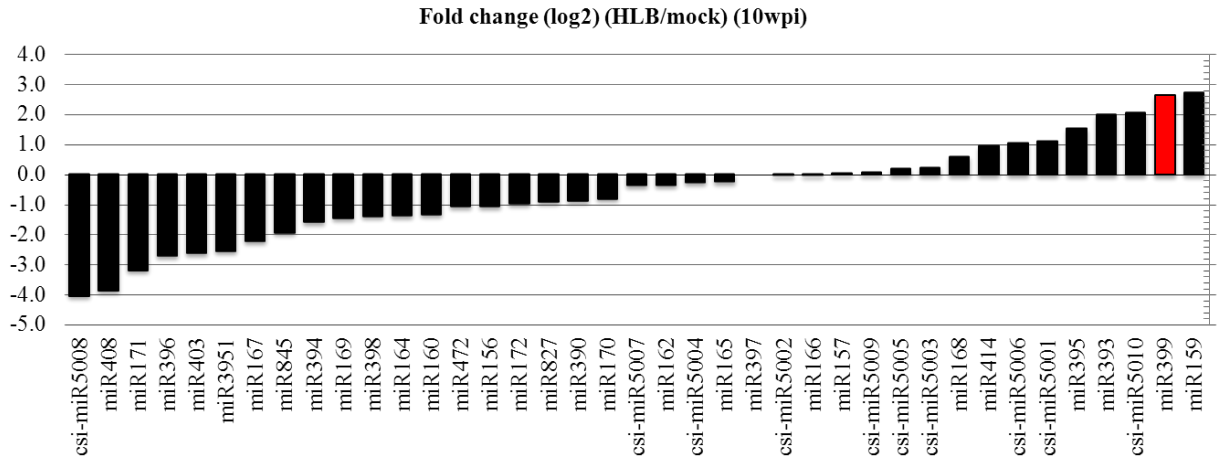
- Source-sink disruption
  - Sucrose & Starch metabolism
- Immune disfunction
  - Inappropriate hormone cross-talk
- Protein misfolding stress

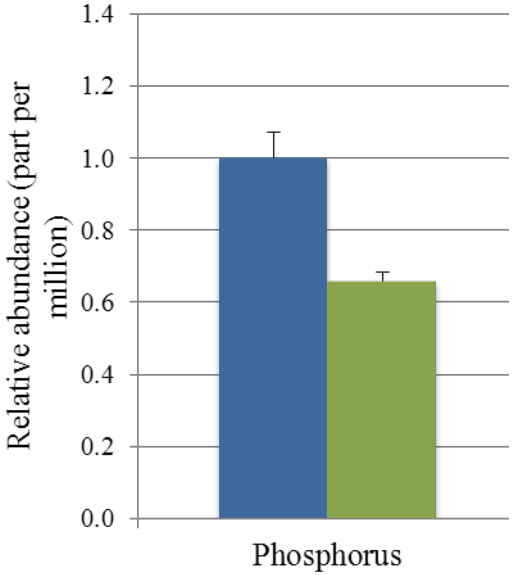


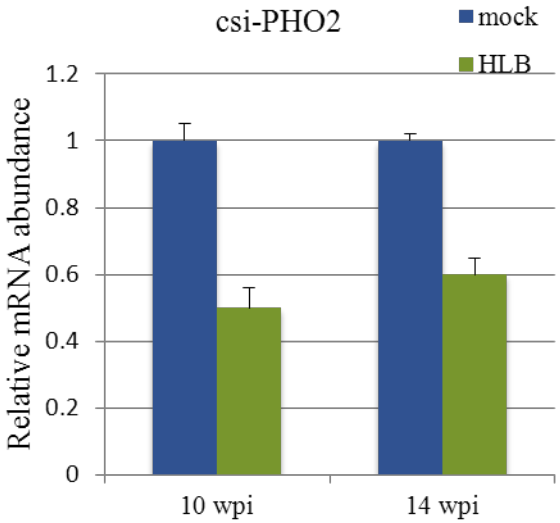
Martinelli et al., 2012. PLoSONE 7(5): e38039

Regulation of small RNA during HLB infection

- Small RNA were identified by sRNA-seq
- sRNAs are regulated during CaLas infection
- CaLas infection of citrus causes P and iron deficiency
- Down regulation may be mediated by sRNA

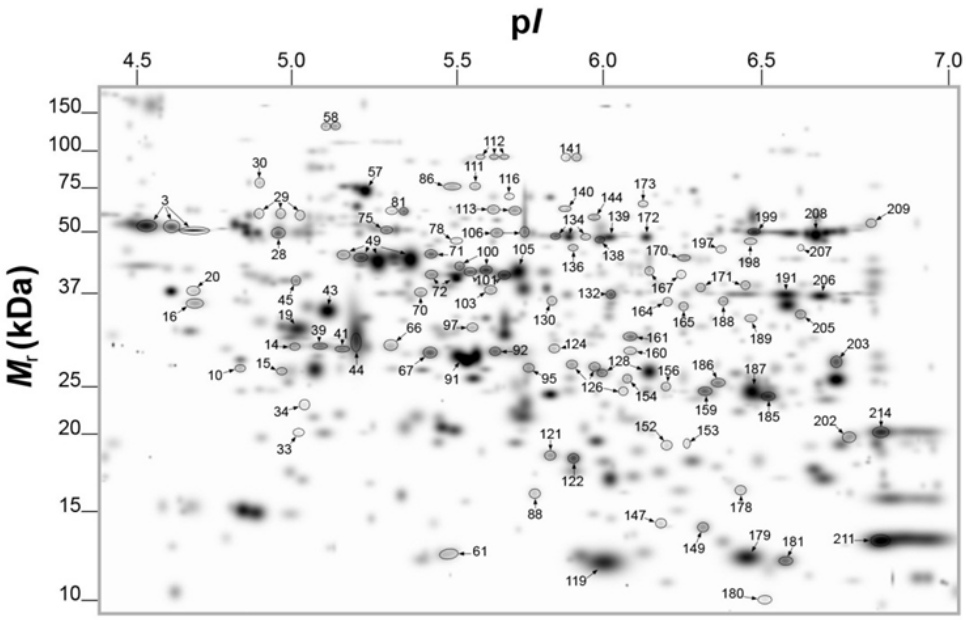


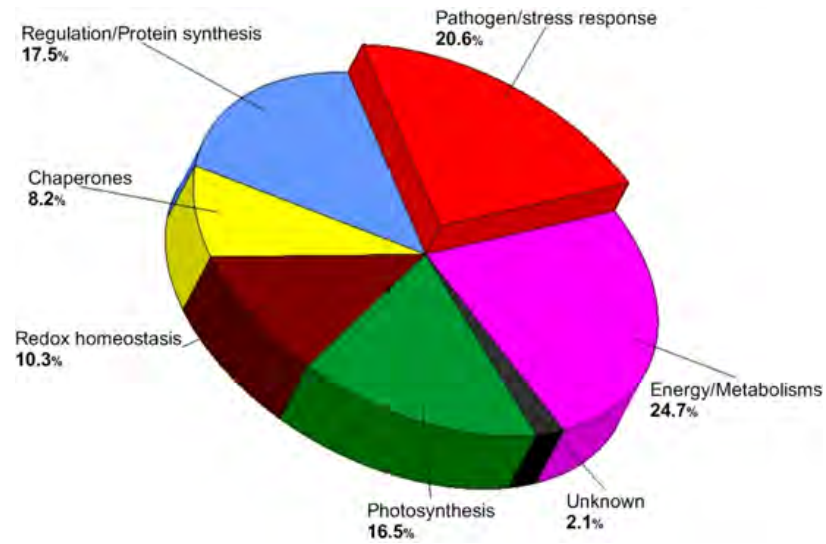




Hailing Jin Laboratory

Proteome analysis of CaLas infected citrus leaves





Hong Lin Lab 2012

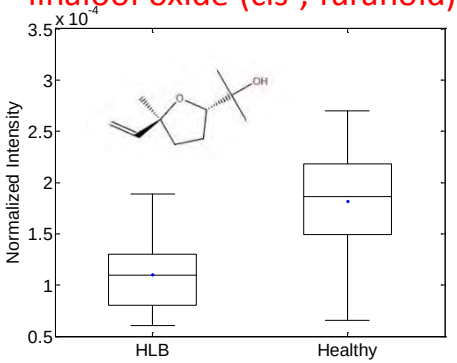
- 686 unique proteins identified by iTRAQ in mature leaves
- significantly, up-regulated proteins
  - stress/defense response
  - four miraculin-like proteins, chitinase, Cu/Zn superoxide dismutase and lipoxygenase

Fan et al., 2011. Physiol. Plant. 143: 235–245.

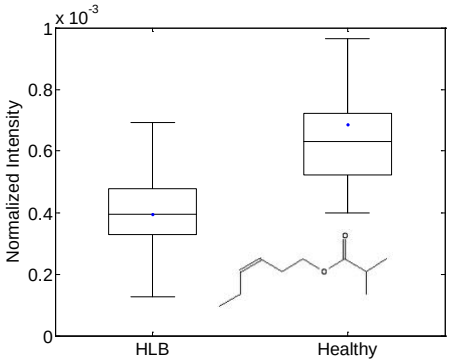
Metabolomic Analysis of HLB infected Tissues

- Metabolite profiling of infected Citrus leaf tissues
  - Metabolites by GC-MS
  - Metabolites by NMR
  - VOCs by GC-DMS, GC-MS
- GC-DMS - Changes in
  - monoterpenes,
  - jasmonates
  - methyl salicylate
- NMR - Changes in citrus fruit
  - Amino acids
    - Decrease in pro, arg
    - Increase in Phe
  - Organic acids
    - Increase in citrate
  - Sugars
    - Decrease in Sucrose
    - Decrease in Glucose

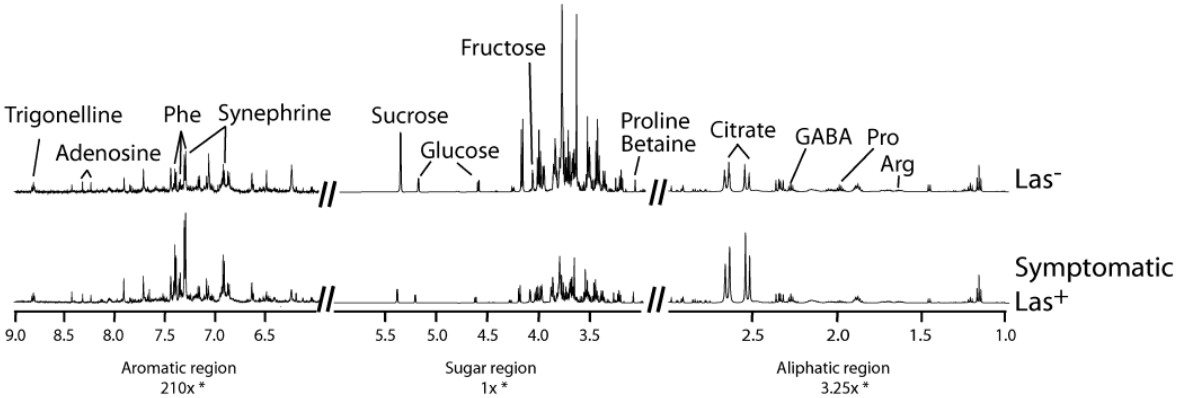
linalool oxide (cis-, furanoid)



hexenyl isobutanoate (3z-)

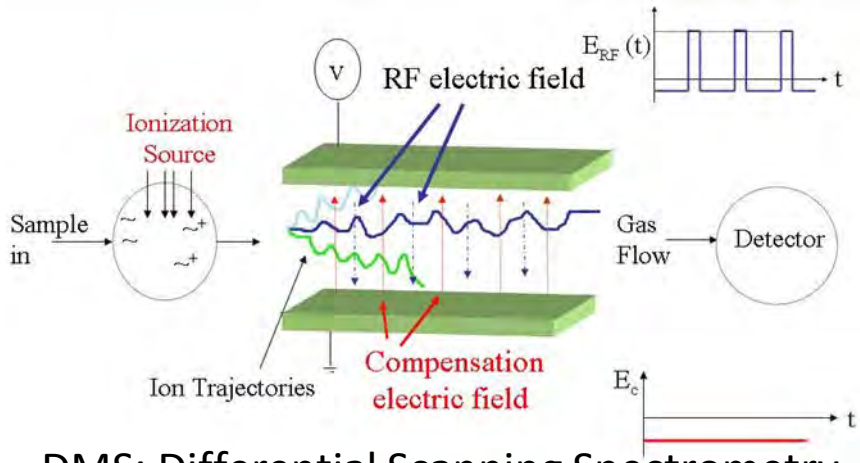


Cristina Davis and Oliver Fiehn laboratories



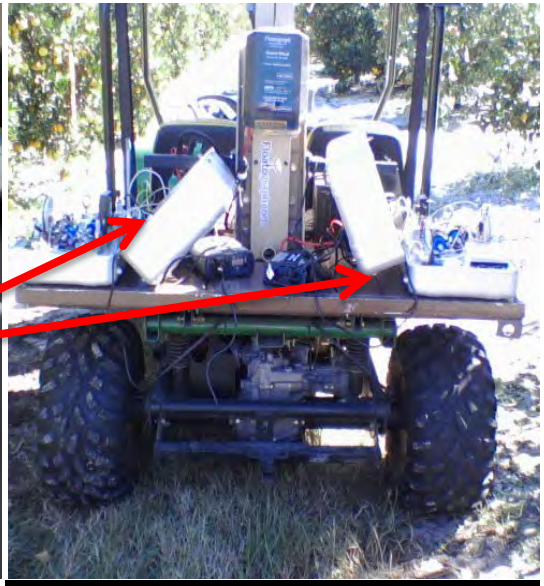
Slisz et al., 2012. J. Proteome Res. 11: 4223–4230

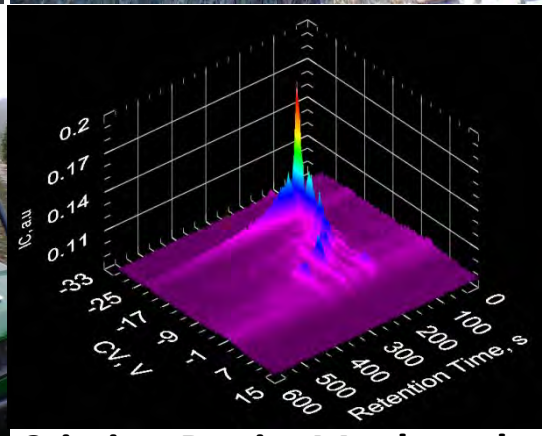
Field testing of VOC sensor technologies for early host response phenotyping.



DMS: Differential Scanning Spectrometry

- An artificial nose sensitive to below picomolar levels
- Robust
- Reagentless
- Software upgrades for new applications
- Handheld versions
- Low cost-of-ownership
- Minimal maintenance





Cristina Davis: Mech and Aerospace Eng; UC Davis

ANI: Advanced Nanotech Inc., Austin Tx

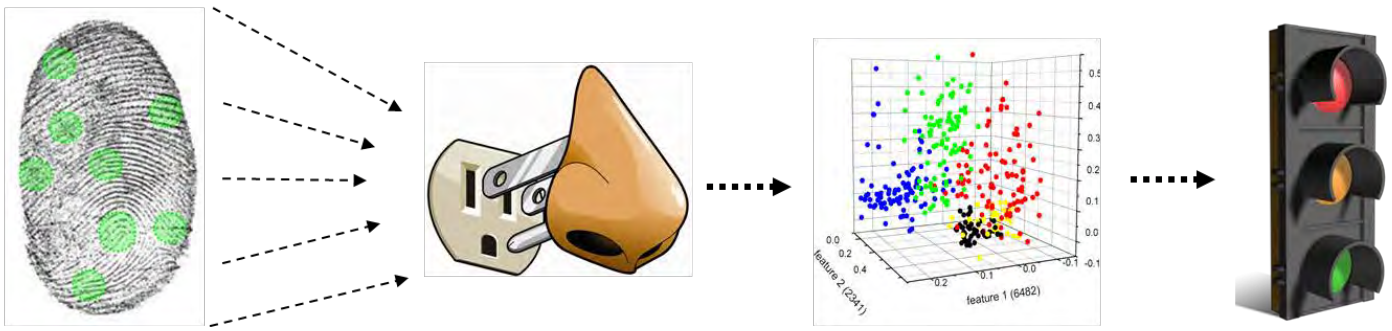
Non-Destructive Phenotyping/Fingerprinting of Host Response BVOCs for Citrus Disease Detection

Chemical Compounds Or Biomarkers

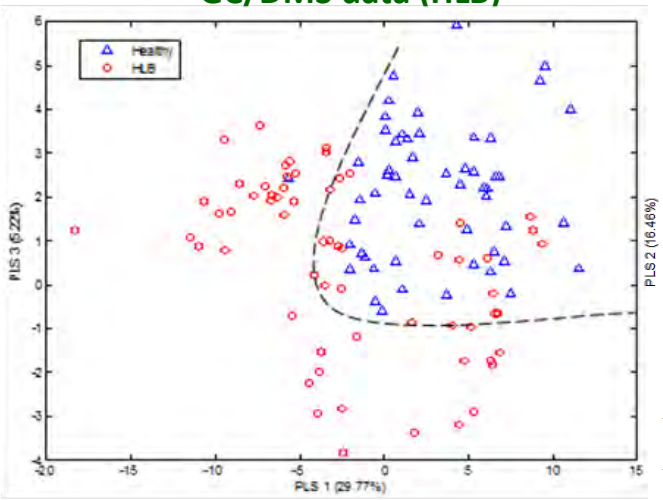
Instrumentation Measures Chemical Signals

Reduced Signal Space By Pattern Recognition

System Output

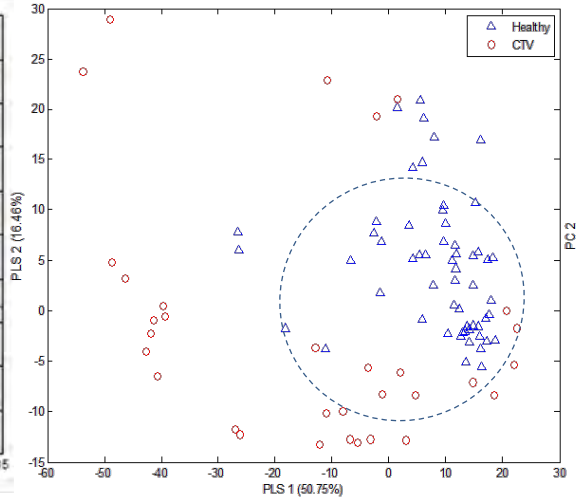


GC/DMS data (HLB)



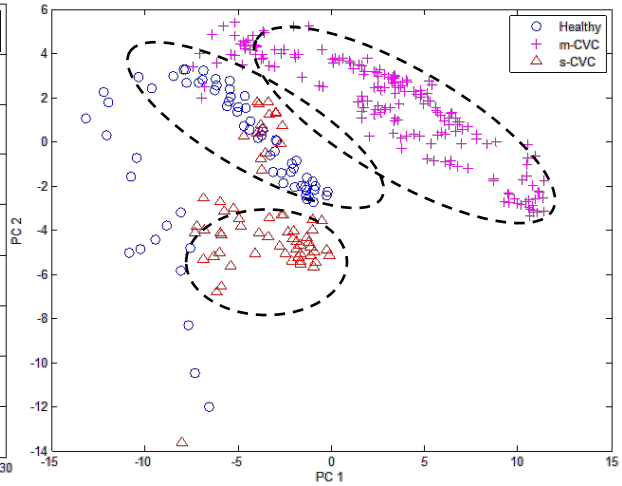
Accuracy: >80%

GC/DMS data (CTV)



Accuracy: >95%

GC/DMS data (CVC/greenhouse)



Accuracy: ~80%

Data generated by Dr. Alexander Aksenov, Dr. William Cheung; analyzed by Dr. Weixiang Zhao.Cristina Davis Lab

Is there variability in HLB disease incidence/severity among commercial citrus cultivars?

Surveys of commercial groves

- revealed differences in CLas titer and HLB incidence/severity among mandarins/mandarin hybrids
- Stover and McCollum, HortScience 2011

Sunburst

Minneola

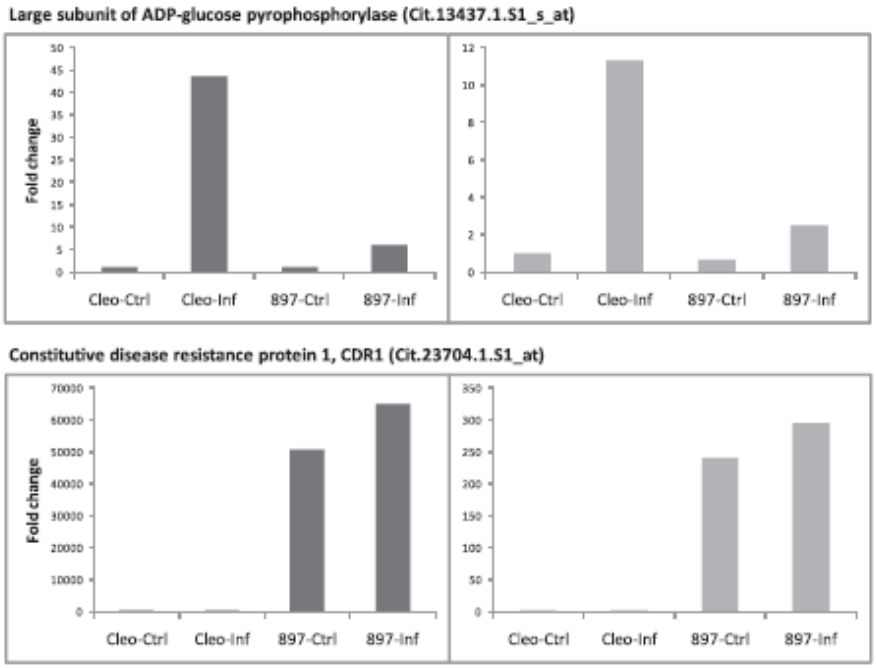
Greenhouse studies

- 16 citrus genotypes (mostly commercial cvs.)
- Inoculations by free-ranging ACP / CLas inoculum source
- CLas titer determined over time (35 weeks)
- Kinetics of CLas titer differ among genotypes
- McCollum, Hilf, Irey unpublished results

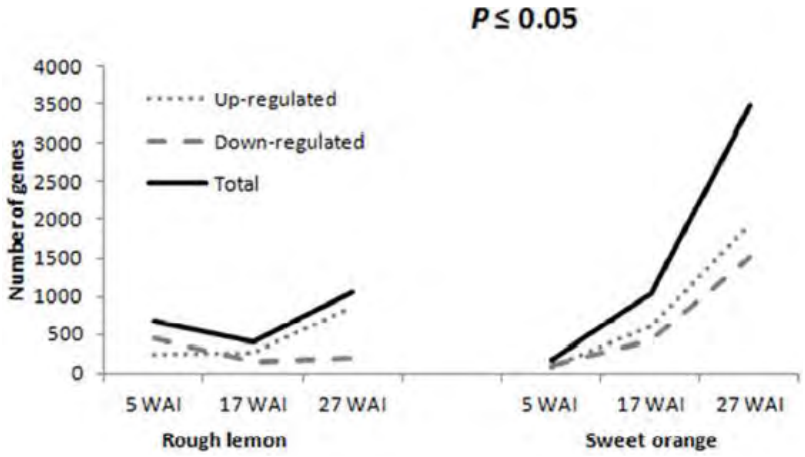
McCollum Laboratory

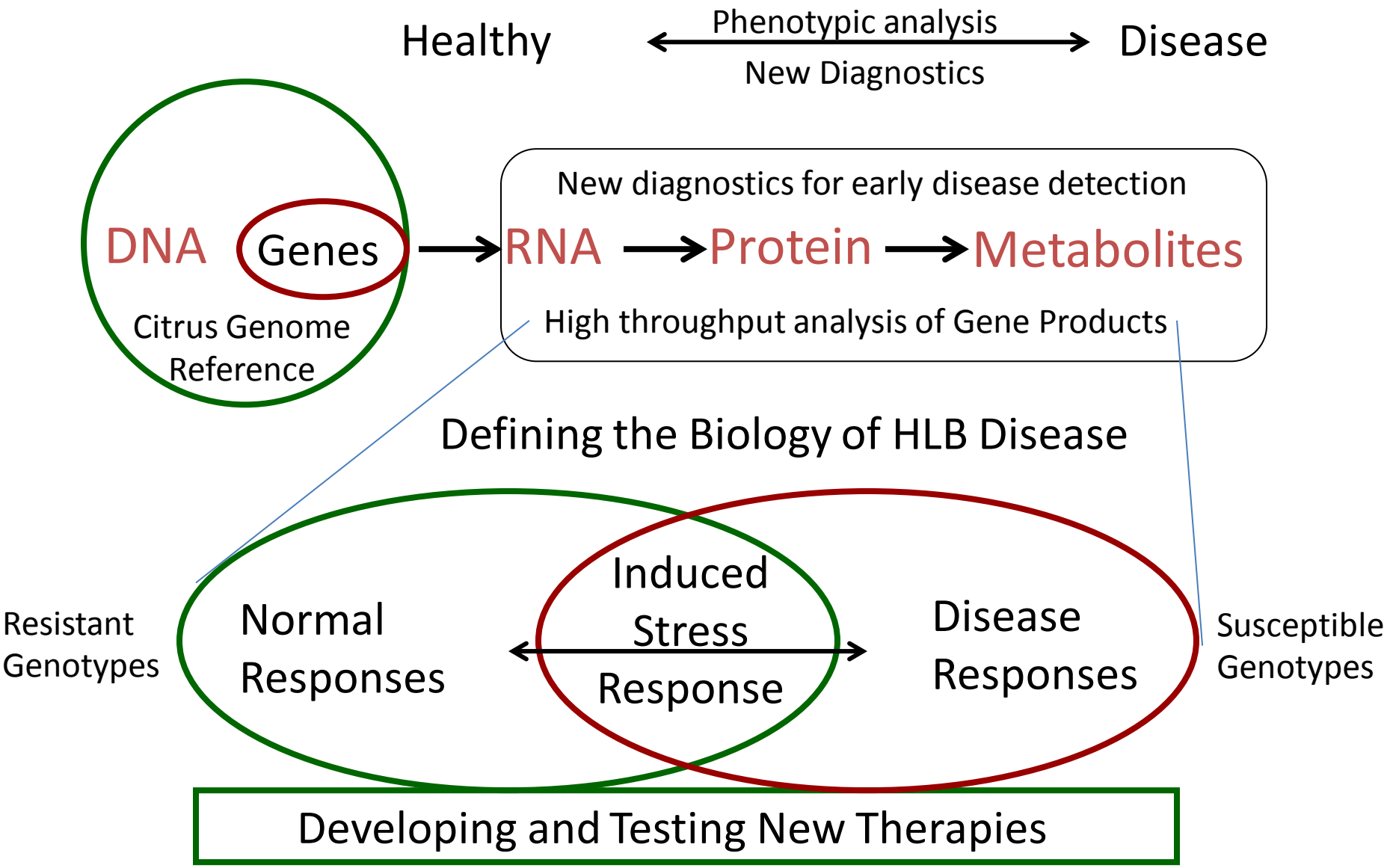
Microarray analysis of tolerant/resistant citrus genotypes

- Analysis of mechanism tolerance of US-897 to HLB – Comparison with Cleopatra mandarin seedlings
  - 326 vs 17 genes up-regulated in US-897
  - Increased disease resistance pathways
  - pp2 expression not significantly changed
- Comparison of Rough Lemon to Sweet orange
  - Higher levels of genes expression in sensitive sweet orange
  - Phloem transport was less affected in rough lemon although callose deposition was observed
  - No phloem collapse was observed
  - Root system of rough lemon was unaffected while sweet orange was severely degraded
  - -pp2 expression not significantly changed



Albrecht and Bowman 2011. Plant Sci.



Fan et al., 2012. MPMI

Functional Mapping of Host Responses