Needed by the citrus industry

(esp. Florida)

Conditions and materials to support replanting with confidence (It's the profit that matters)

Trees highly resistant to Clas
Trees highly resistant to ACP as well
(or Non-CLas-transmitting ACP)

If resistance is not robust,
better diagnostics are needed to find infected trees

Koch’s postulates

Duan and others have made a convincing case that economically important HLB is caused by Clas

but Kochs postulates, or even the long-term culturing of Clas, have not been accomplished

(thought poached from Ed Civerolo)

Outcome Group 1
Keep groves productive

Needed for ACP control

AWPMP and CHMA

New spray technologies and chemistries

Combat development of insect resistance

Needed for tree Health

New experiments for deconvoluting current nutritional mixtures

New types of “rational nutritionals”

based on predictions from transcriptomics and metabolomics

(this notion derived from a remark by Abhaya Dandekar)

Outcome Group 2 prevent or slow ACP and CLas spread

Diagnostics and biomarkers for early stage infection

Requirements and validation

(for early stage detection, even greater demands)

- Accurate
- Sensitive
- Specific
- Reproducible for
  - Season, climate, time of day
  - Citrus species and varieties
  - Locale/soil
- High throughput

Approaches

- Analysis of extracts (difficult to make high throughput)
  - Antibody-based
  - Nucleic acid-based
  - Small molecule reporter)
- Optical (multispectral, fluorescence, polarization, spray reporter)
- **Volatile organic compounds (VOCs) (esp. high sampling volume)**

Diagnostics and biomarkers for early stage infection

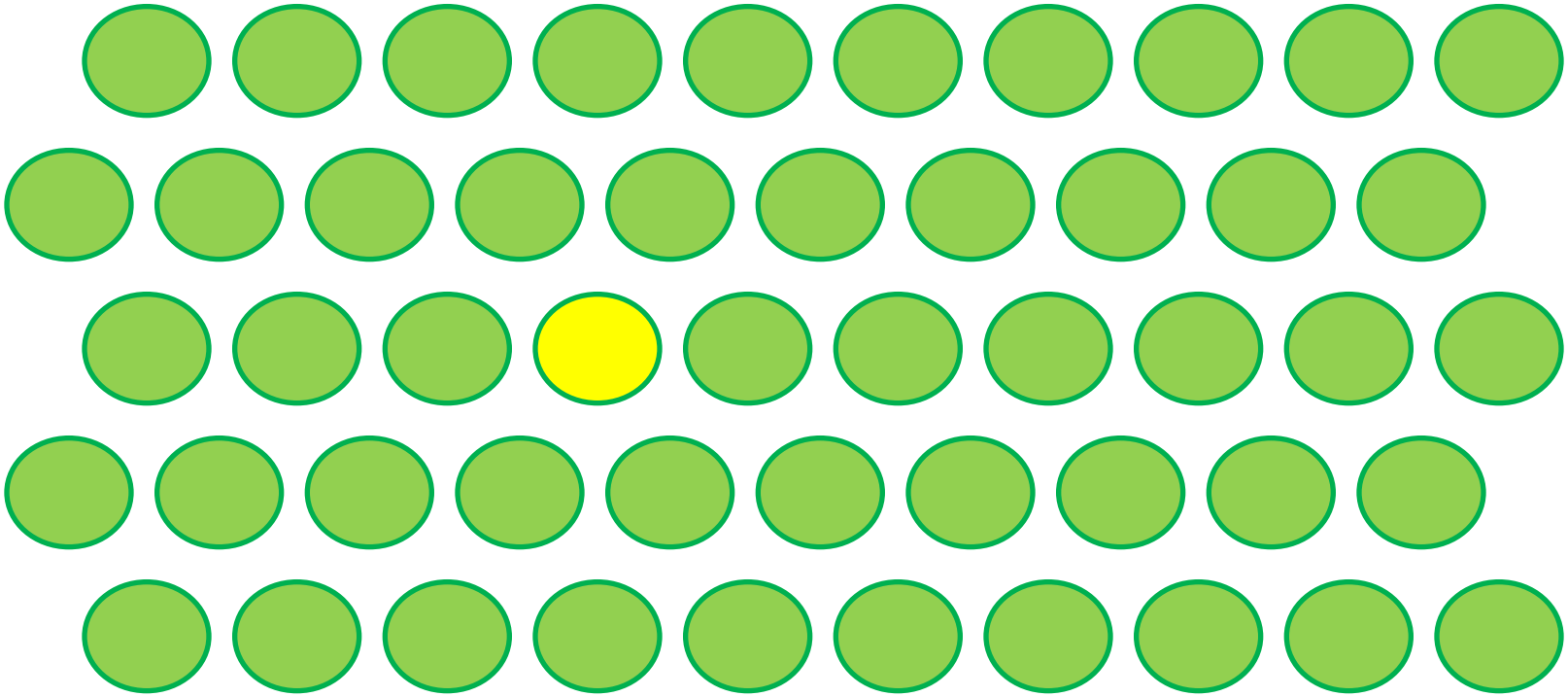
Approach with great promise

Volatile organic compounds (VOCs)

For VOCs

Biomarker discovery and VALIDATION (time and space)

<u>High volume and high throughput sampling</u> to find a single, asymptomatic but infected tree among many

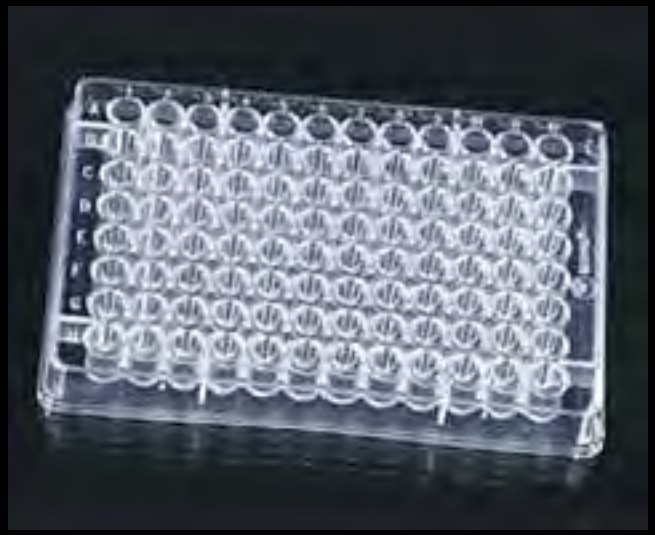


ACP trapping and ACP distraction (OG2,3)

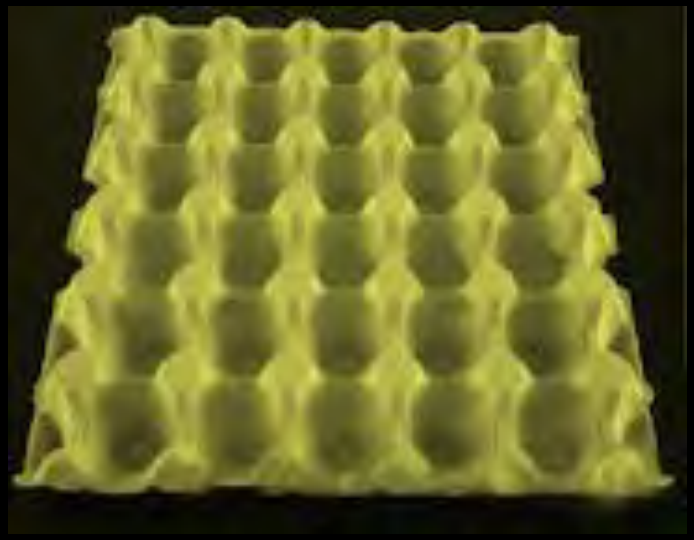
New semiochemicals, discovery and development
SPLAT and other delivery technology for volatiles

Currently known natural attractants are not powerful
Therefore, turn to synthetic chemicals

Chemical combinatoric approach to semiochemical discovery
(library amounts of combinatoric chemicals are very limited)



Candidate chemicals from library to segmented trap



Orchard test

Related to Inscent, Inc. approach but simpler and much less sophisticated (no direct measurements of interaction with insect organs or molecules; just count insect bodies)

Outcome Group 3 Culture citrus trees free of ACP and CLas

Main approaches

Vectoring-debilitated ACP

Resistant citrus

Conventional, transgenic (scion and/or rootstock)

Conventional selection: encouraging results (significant grove variation in susceptibility)

Transgenic: It appears that the research threads and results have been developed to create CLas-resistant, and perhaps ACP-resistant, transgenic citrus cultivars or citrus endosymbionts.

Weaving the threads into a functional fabric will require time, money and surmounting technical and IP hurdles.

Transgenic, resistant citrus

Weaving the threads into a functional fabric will require time, money and surmounting technical and IP hurdles

CLas resistant citrus

- **AMPs, defensins and other antibiotic peptides**
- **Other anti-CLas small molecules**
- **Engineered proteins for CLas lysis**
- **Production of CLas quorum sensing compounds**

ACP-resistant citru

- **AMPs, defensins and other insecticidal peptides**
- **Psyllid-active Bt**
- **Anti-psyllid RNAi**

Ideal resistance mechanism and strategy:

Stack genes to provide multiple (e.g., 2 from each list above) and distinct mechanisms aimed at the same target (strategy for achieving durability, i.e., resistance for the life of the cultivar)

Rootstock-delivered if possible

Transgenic, resistant citrus

Because of the dire consequences HLB spread, circumstances for deploying transgenic citrus with public acceptance have become more favorable

(Cf the dire situation for papaya and PRSV in Hawaii in the 1990s)

Threat of the loss of an entire industry from the US (orange juice)
Significant domestic market for the transgenic product
Grower groups accepting of new technology

We heard general acceptance of transgenic technology from the grower panel

This rational attitude by growers may not be readily transferred to a general public stirred up by activists, but the above considerations are very favorable to public acceptance

Closing remark: It is often stated:

"If it ain't broke, don't fix it"
This is now an outdated statement.
The more effective (but clumsy) objective is "to fix it at some time before it otherwise would break"

Jet engines and other complex devices are instrumented to detect developing faults that otherwise would result in failure at some point in the future

New pathogens and revisits by old pathogens will always be occurring

Anticipating such an event (e.g., introduction of citrus variegated chlorosis) and acting to prevent it or manage it has great potential to save money and time