Characterization of Liberibacter populations and development of field detection system for citrus huanglongbing

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NIFA annual meeting – Feb 17, 2016
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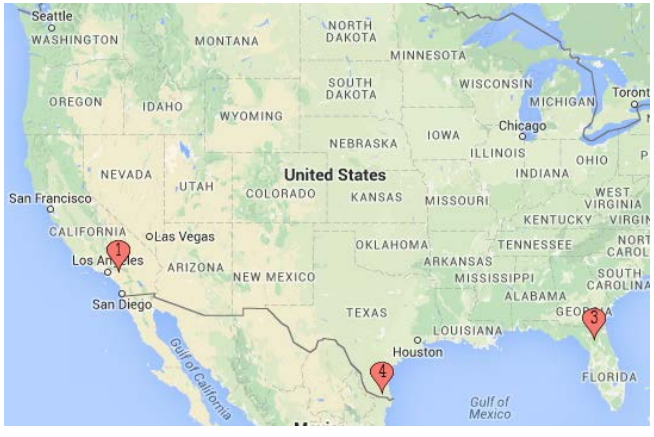

http://liberibacterdetection.ucr.edu/

Project participants

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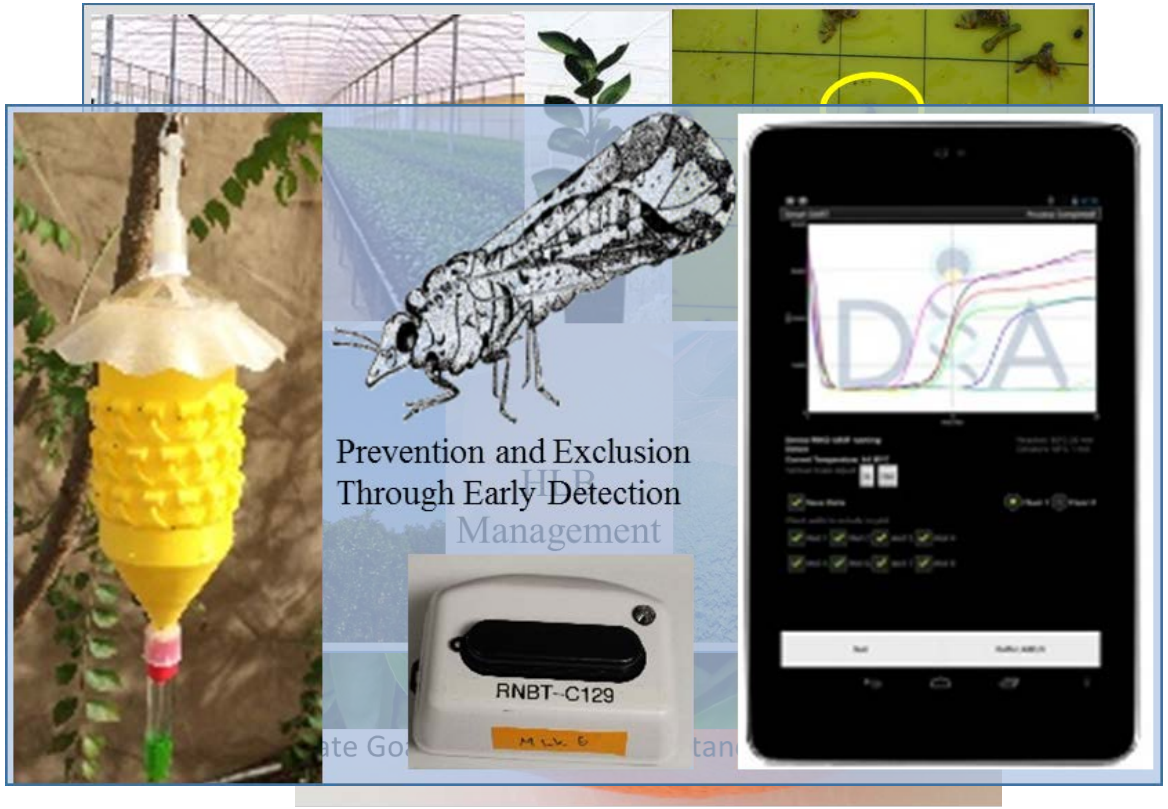
Citrus is historically very important for California and for Riverside

Riverside has one of the two original trees of Parent Washington navel obtained from Bahia, Brazil in 1873. Most navel oranges in the country and elsewhere are clones of this tree.

Riverside county was the richest in the country at one time because of citrus.

Citrus experiment station started in Riverside in 1907. 50 years later, a University was started (UCR).

UCR’s Citrus Variety Collection has 1200 accessions obtained from all over the world.



Protecting the Citrus Industry from HLB

APHIS

cdfa

CALIFORNIA DEPARTMENT OF FOOD & AGRICULTURE

USDA ARS

CA was pro-active in HLB management.

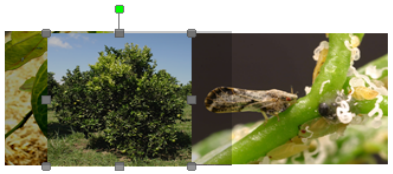
Detailed planning to exclude ACP and HLB since 2006.

***Huanglongbing* (HLB) or Citrus Greening and its Vector, *Diaphorina citri*, the Asian Citrus Psyllid (ACP)**

Draft Action Plan for California

Draft 1: November 19, 2007

HLB Task Force
2007



The California Huanglongbing Task Force Members

Steering Committee

Larry Bezark, CDFA Co-Chair

Ted Batkin, Citrus Research Board, Co-Chair

Elizabeth Grafton-Cardwell, University of California
MaryLou Polek, CDFA
Wally Ewart, California Citrus Quality Council
Chuck Orman, Sunkist Growers

Helene Wright, USDA, APHIS
Edwin Civerolo, USDA, ARS
Joel Nelsen, California Citrus Mutual

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MaryLou Polek, CDFA, Chair

Elizabeth Grafton-Cardwell, University of California
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Glenn Wright, University of Arizona

Kris Godfrey, CDFA
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Pam Geisel, University of California
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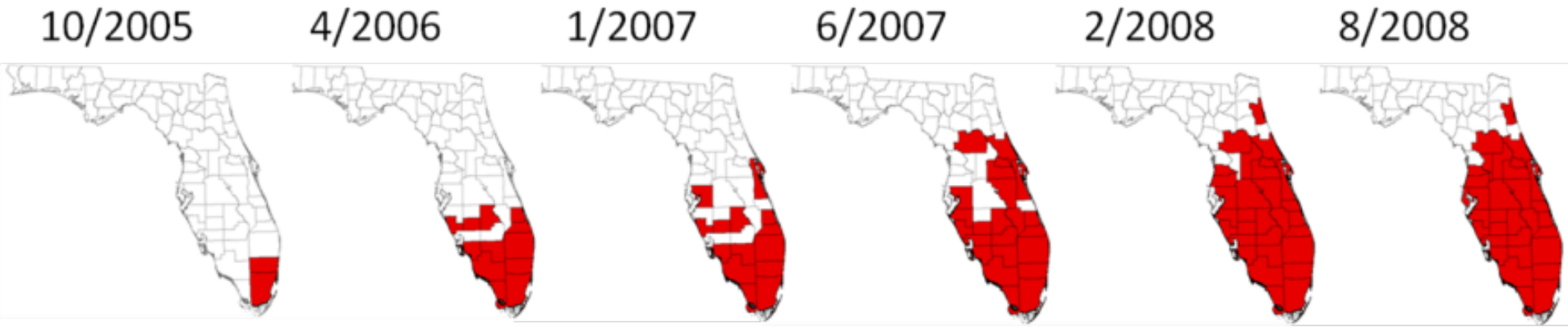
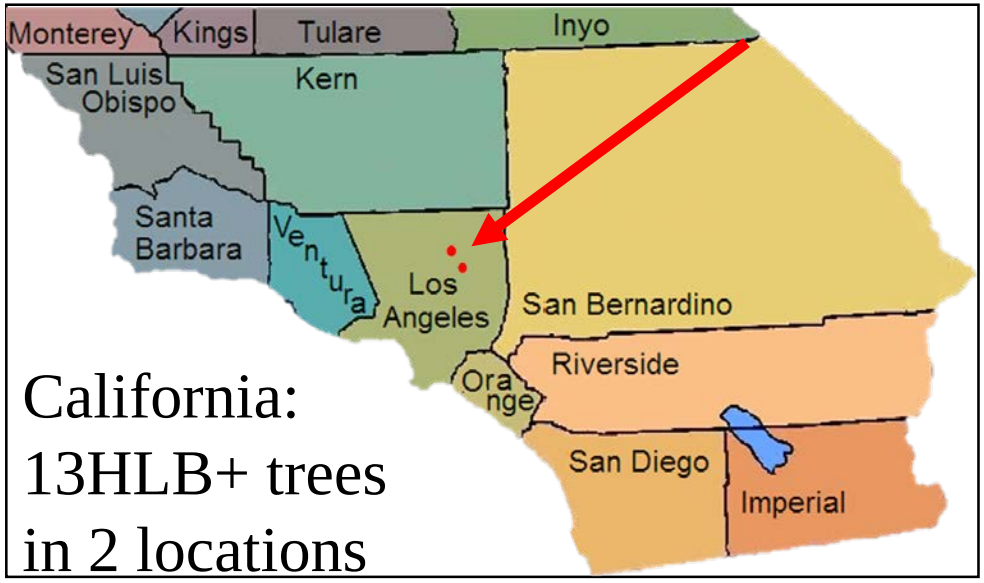
Larry Hawkins, USDA, APHIS
Kris Godfrey, CDFA

Anne Warring, CA Citrus Research Board
Jay Van Rein, CDFA
Magally Lugae-Williams, CDFA
Jerry Prieto, Fresno County Ag Commissioner

Compare the HLB epidemic in three states (3 years after the first report)



HLB in every major citrus growing county



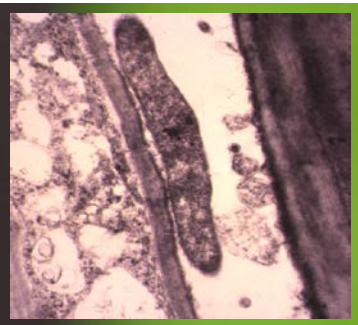
California HLB situation

- Plants from the two HLB positive find sites were analyzed and found to be two separate introductions.

- It is likely that the Hacienda Heights strain that was reported in 2012 was ‘contained’.

- Genome sequencing is important for knowing about the spread, type of pathogen/strain that is being spread, efficacy of disease management measures. Are we doing enough?

- Sequence information is also useful for developing assays to detect all variants of the pathogen.



Garnier

In a low titer situation, HLB bacteria may be acquired by psyllids leading to rapid multiplication

Photo by Michael Rogers


Different scenarios of
sampling:
Plant vs psyllid

Finding HLB pathogen in psyllids can give us an early warning

County	Psyllid record	Plant record
Brevard	May 2006	Dec 2006
Pasco	May 2006	Oct 2007
Marion	June 2006	May 2007
Polk	Sept 2005	Aug 2007

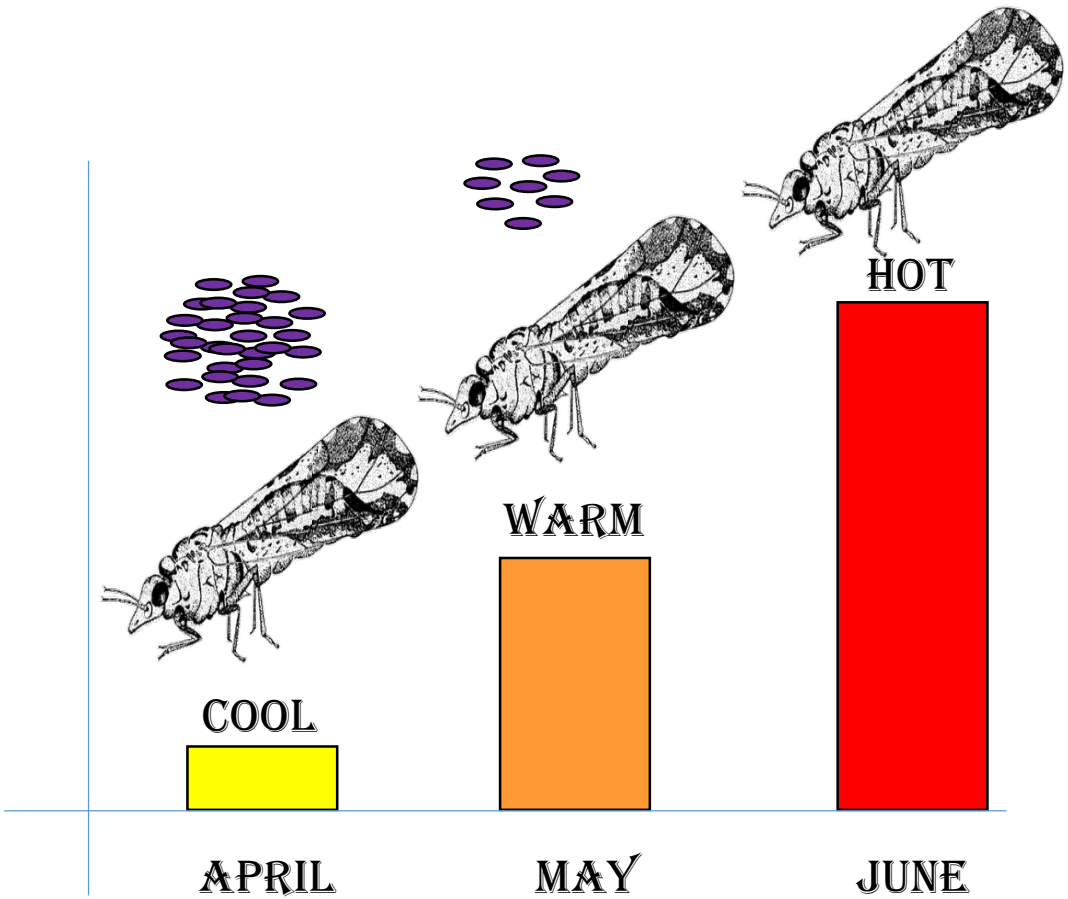
Data from Keremane et al., 2008 Phytopathology 98: 387-396.

- In California, Liberibacter positive psyllids have lead to finding HLB positive plants.

- The best sample to test first is the psyllid.
    a) test psyllids
    b) test psyllids more often.

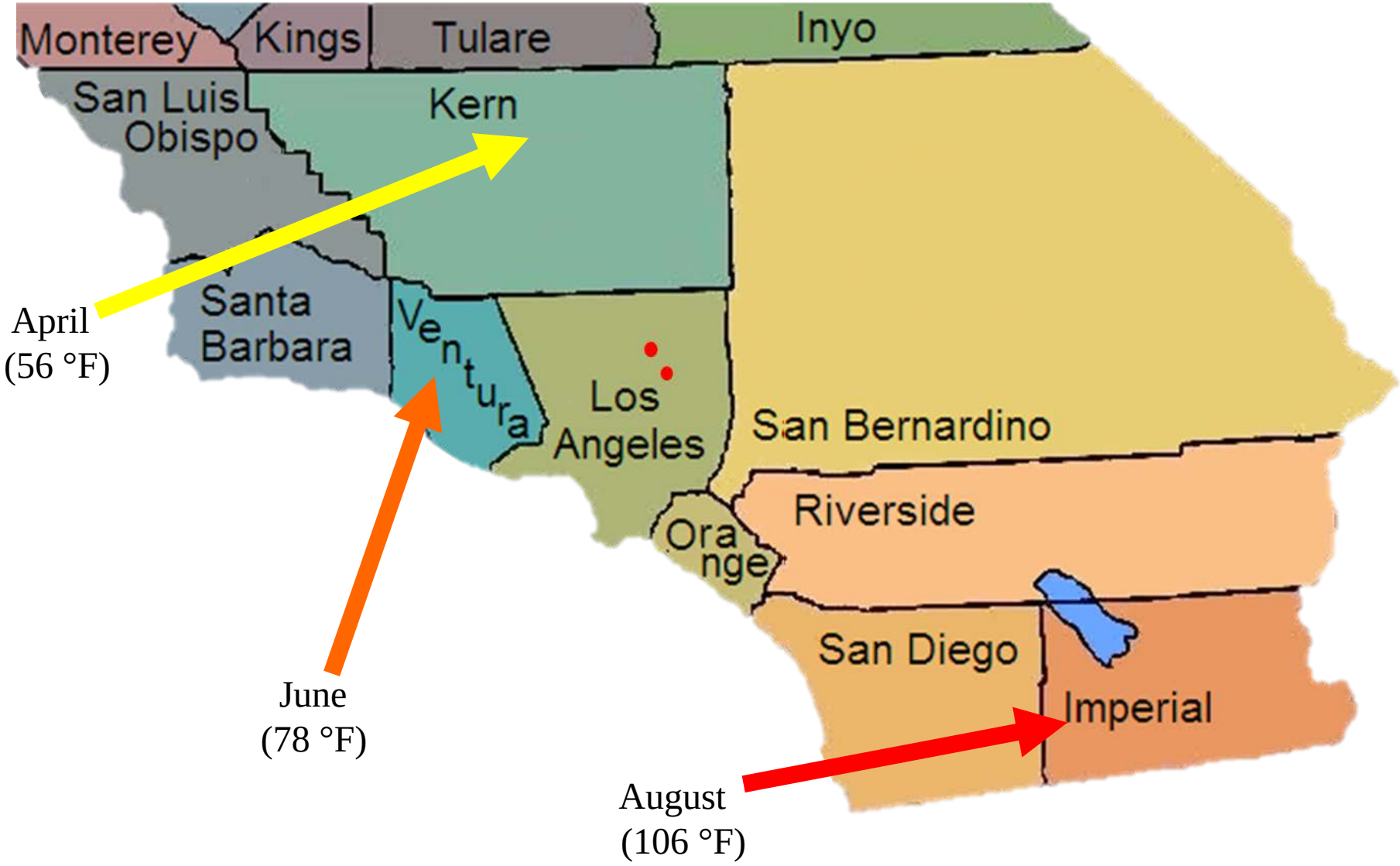
- Sample psyllids adequately and correctly.

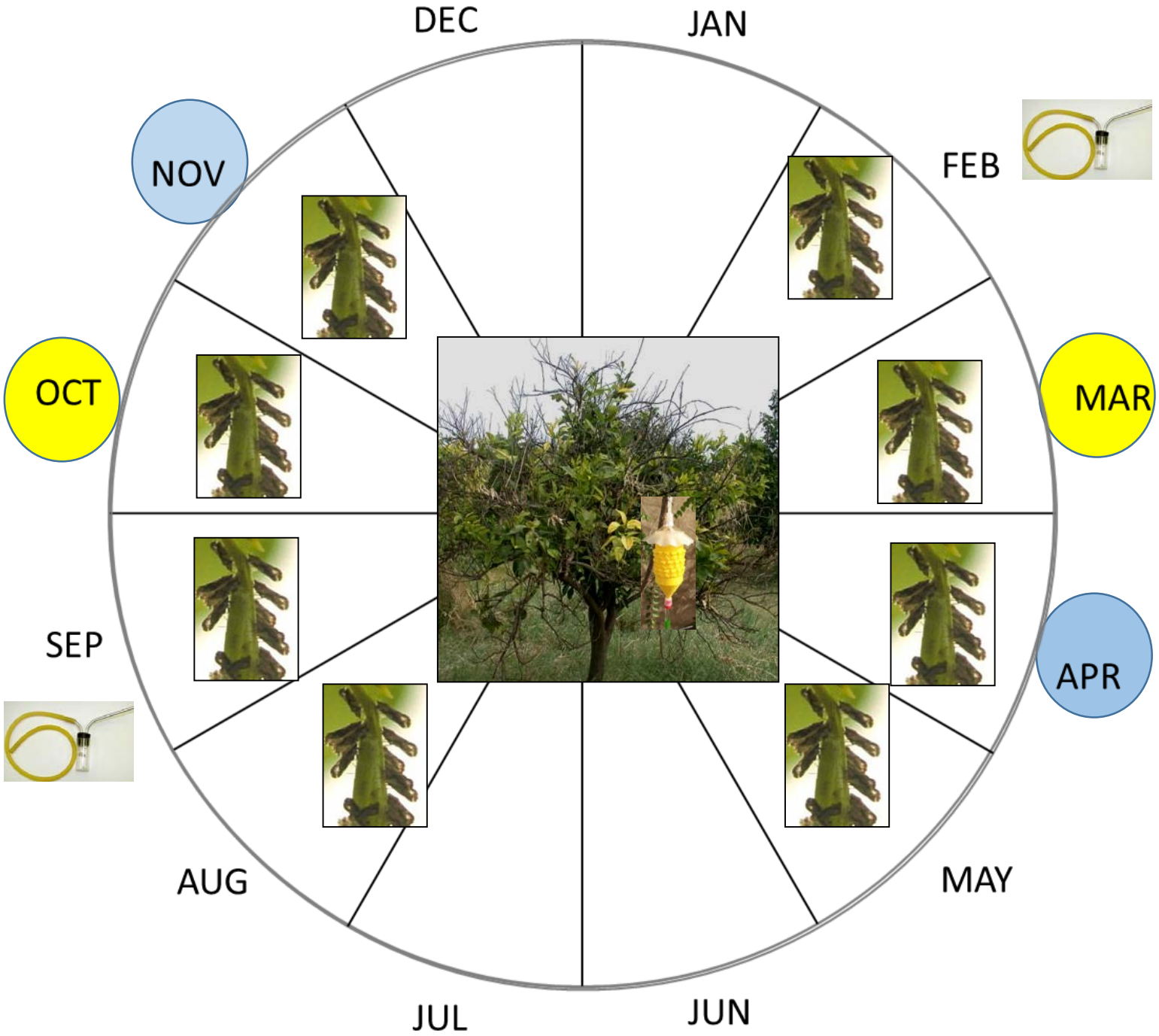
Psyllids do not carry the pathogen at all times



Data from Pakistan: Razi et al., 2014, Phytopathology

Is the sample collection done under optimal conditions?




DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV

Advantages of Smart traps

1. Dynamic sampling as opposed to static sampling.

2. Psyllids caught are preserved and are testable (unlike yellow sticky traps).

3. Can cover more area, collect at different times.

4. Improvements are being made to increase the capture rate.
   - use of LED lights.
   - use of attractants.

SmartTraps designed and constructed by the DPI Florida

Evaluation of SmartTrap Models

Pomona, CA. Evaluation in cooperation with USDA APHIS & CRB

Testing for Liberibacter

- Approved method of testing is by qPCR.
- Large number of samples need to be tested throughout the year – especially at the start of a disease epidemic.
- Need “many eyes” looking for HLB. Involve growers.
- Growers can use on-site detection methods.
- To fulfil this need, we developed field detection methods.

Field detection system

- Isothermal loop-mediated amplification technology (LAMP).
- Field testing. If samples are positive, further validation will be needed.
- This would complement the prevention and exclusion efforts by Government agencies.

Smart-DART™ platform with handheld device to conduct diagnostic assays using isothermal nucleic acid amplification. Bluetooth interface is provided on a mobile Android device

Bluetooth

Hardware and software from U Hawaii.

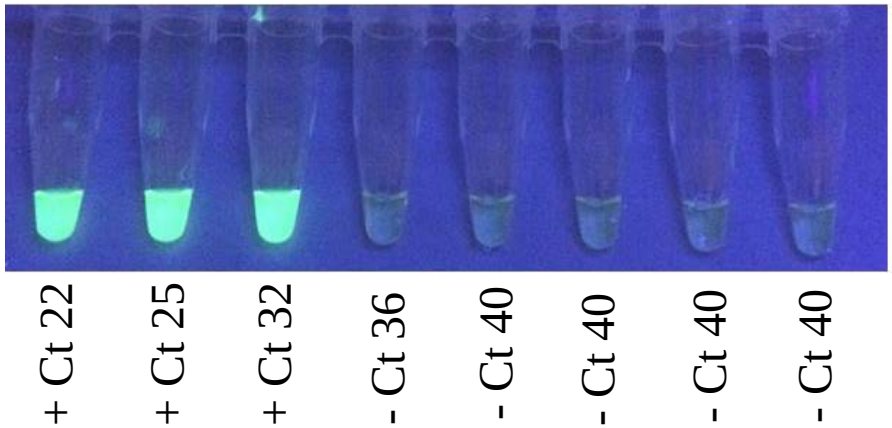
Keremane et al., 2015. Crop Protection 68: 41-48.

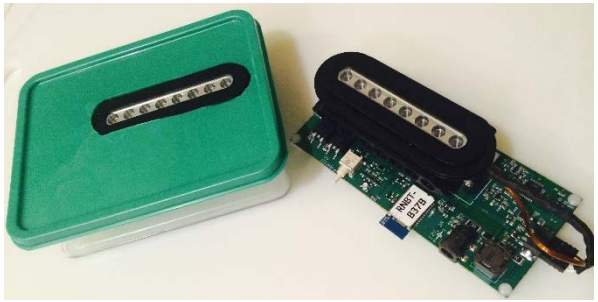
Detect HLB using SmartDart

- After certain modifications to the software and the hardware, SmartDart is working well.
- Cost for purchasing one unit is about $3000. Suitable for large growers.
- Small growers and homeowners will need something cheaper.
- Developing an inexpensive method of ‘Visual PCR’ (2 methods: color change and fluorescence-based).
- Visual PCR is also based on LAMP technology.
- Initial investment will be $300 (instead of $3000).

Less expensive LAMP methods for small growers, extension personnel.

Fluorescence-based LAMP field test for HLB



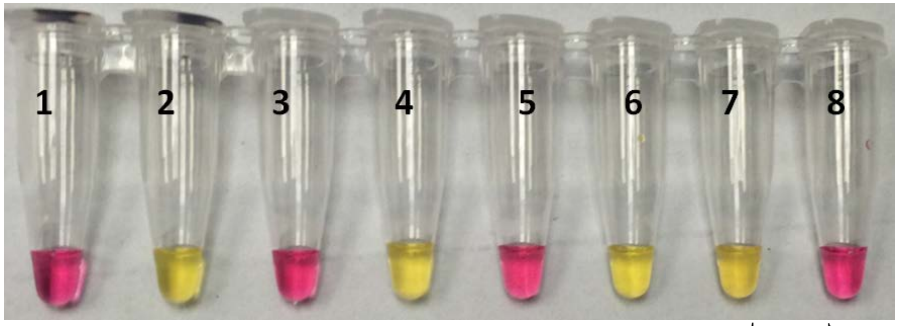


Heating block with long-range UV light $300

Under development

Less expensive LAMP methods for small growers, extension personnel.

LAMP based field test for HLB – visual PCR



Las **-ve** psyllid

Las **+ve** psyllid

Las **-ve** psyllid

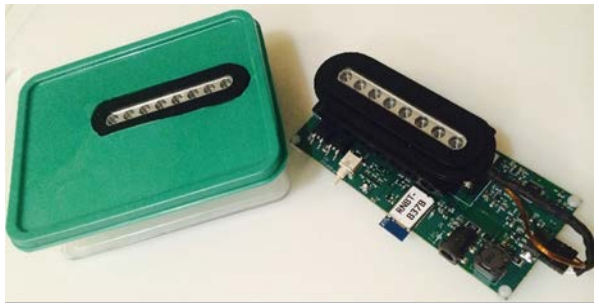
Las **+ve** psyllid

Las **-ve** psyllid

Las **+ve** psyllid

+ Control DNA

Ext. buffer only

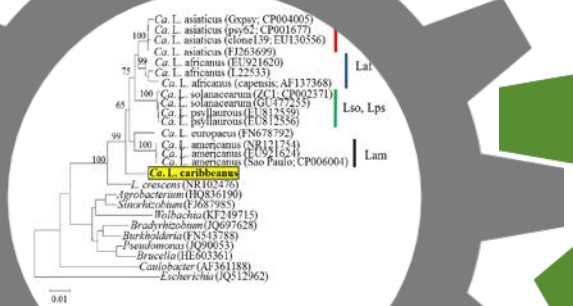


Heating block

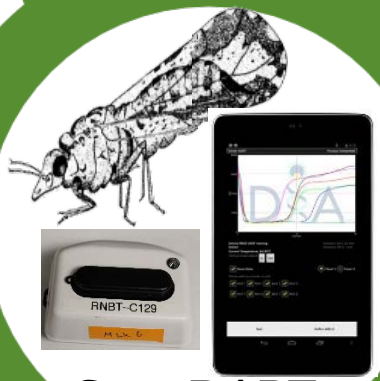
Under development

Smart Trap

Field Testing by Growers



Bacterial sequences



SmartDART

Expected outcomes and outputs of the project

1. The Smart traps that we are developing will enable dynamic psyllid sampling. Testable psyllids will be obtained.

2. Sequence information generated will enable testing of all citrus-associated Liberibacters.

3. Field detection methods will enable on-site testing .

4. Grower involvement will increase the number of people looking for HLB and will lead to better disease mitigation.

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