Recovery Plan Update on Huanglongbing aka HLB / Citrus Greening

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The Pathogen
Candidatus Liberibacter asiaticus (cLas)

- Alpha proteobacterium
  - Gram-negative
- Insect vectored
  - Psyllid-transmitted
- Phloem-limited
- Prophage
  - Plasmid and chromosomally integrated
  - Highly variable regions with frequent recombination and reassortment

- Zhou, LJ, et al. 2013. Prophage-mediated population dynamics of cLas in plant and insect hosts. IRCHLB Poster. 4-7 Feb. 2013, Orlando, FL.
- Zhang, S. et al., 2010. Ca. Liberibacter asiaticus carries an excision plasmid prophage and a chromosomally integrated prophage that becomes lytic in plant infections. doi: 10.1094/MPMI-11-10-0256.
- Zhou, L. et al., 2011. Diversity and plasticity of the intracellular plant pathogen and insect symbiont Candidatus Liberibacter asiaticus as revealed by hypervariable prophage genes with intragenic tandem repeats. doi: 10.1128/AEM.05111-11.

Huanglongbing (HLB) symptoms

The Vector

Diaphorina citri, Asian Citrus Psyllid (ACP)

- Females
  - Need new flush
  - Approx. 500-800 eggs in 2 months
  - Optimum
    - temp 29.6C
    - RH > 40%
  - Lifecycle can be 1+ months

cLas Spread: U.S.

Currently widespread

Florida 2005

Louisiana 2008

South Carolina 2009

Georgia 2009

cLas Spread: U.S.

Puerto Rico
10.06.09

Virgin Islands
04.21.10

Texas
01.13.12

California
03.29.12

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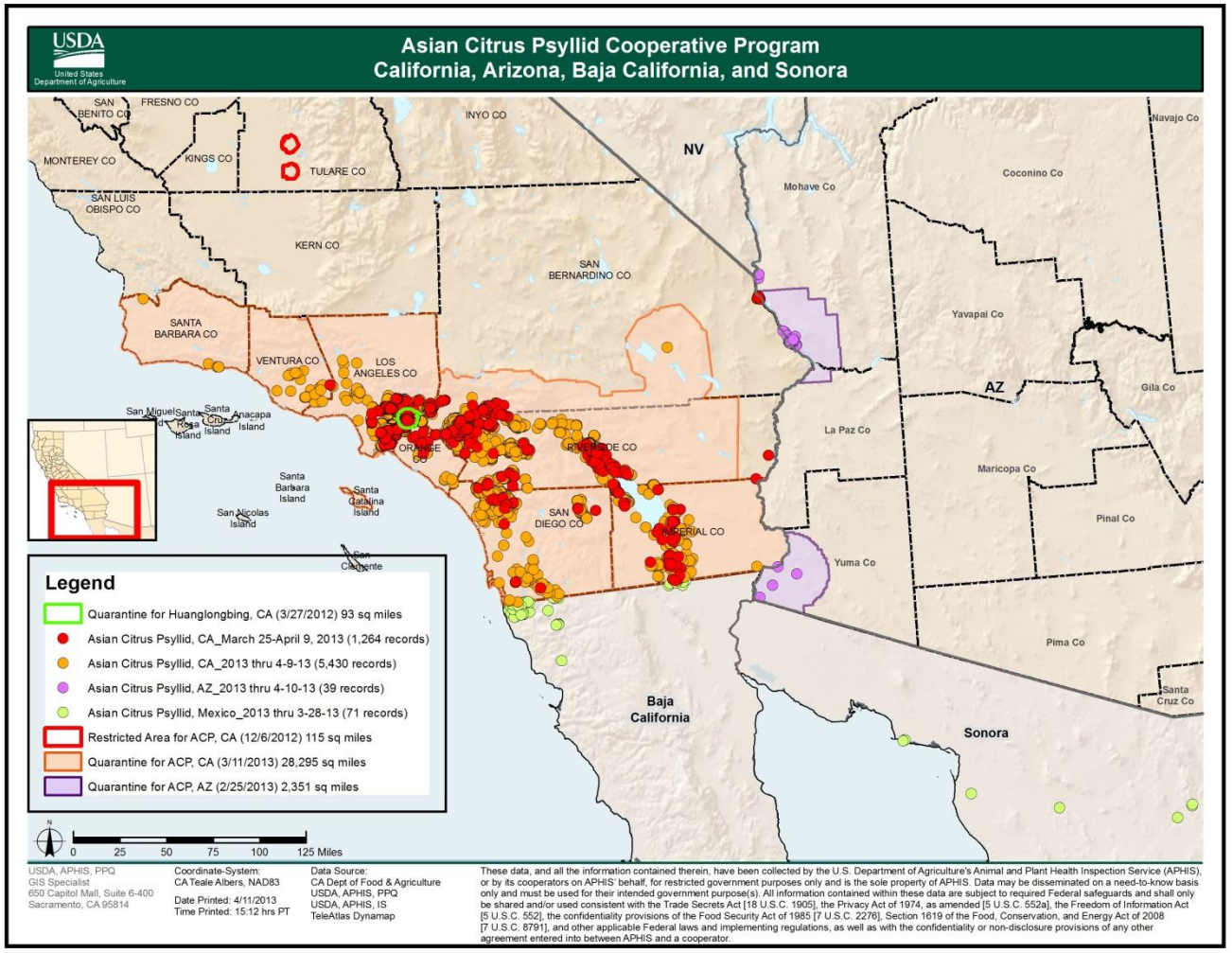
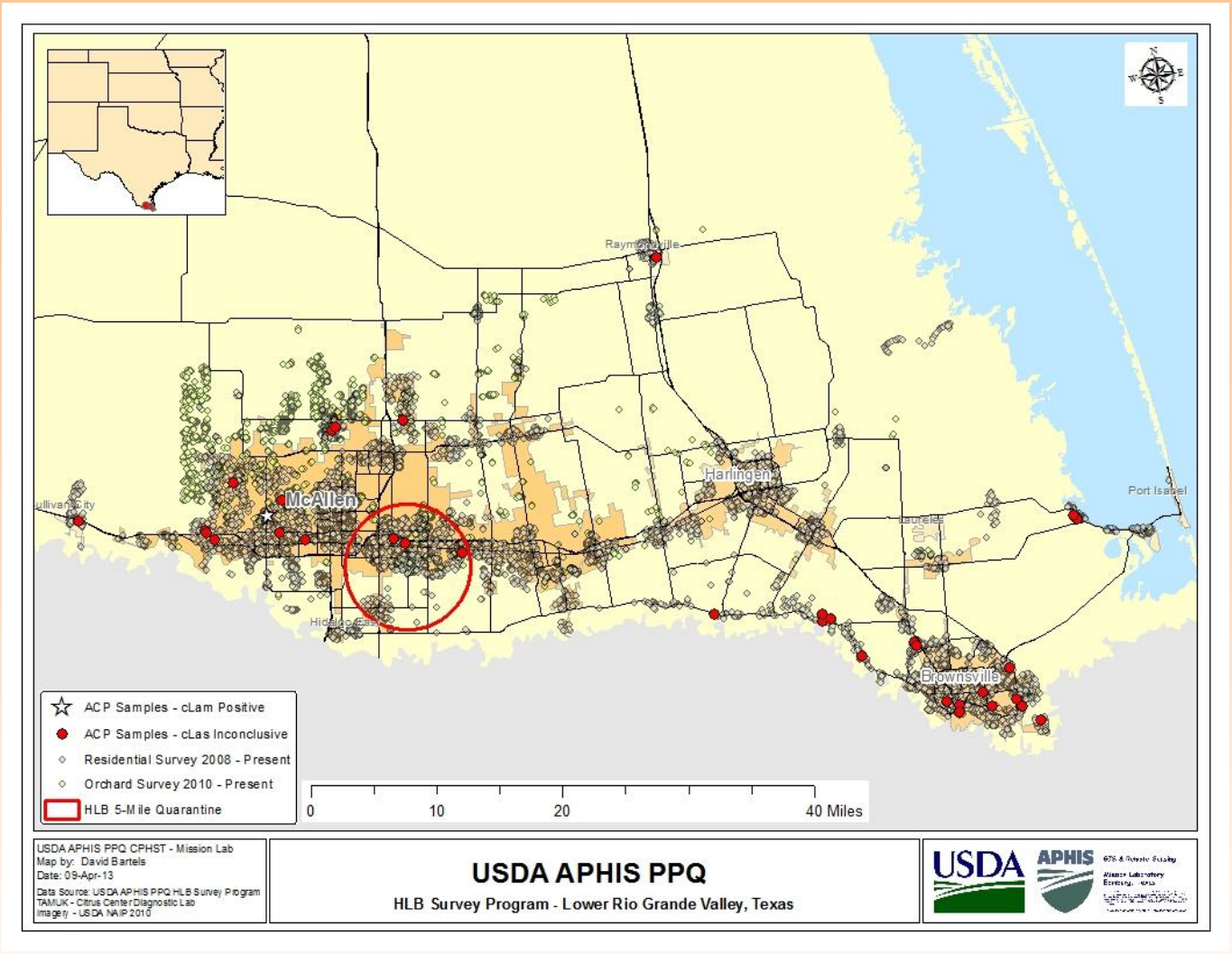
ACP Spread: CA & AZ



ACP Spread: Texas



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Monitoring and Detection

- *Plant volatiles and ACP responses.* cLas infected plant host tissues produce methyl salicylate which attracts ACP
  - ACP prefer feeding on newly diseased plants – sufficiently long to acquire cLas
- Diseased plants are deficient in several nutrients (N, Fe, Zn, P)
  - ACP then disperse to non-diseased plants. Hypothesis: seeking a host with increased nutritional content
- Aged, diseased plants are not as a rule attractive to ACP
  - Attraction is increased by applying plant nutritional supplements

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Stelinski, LL et al. 2013. Induced release of a plant-defense volatile "deceptively' attracts insect vectors to plants infected with a bacterial pathogen. IRCHLB Poster. 4-7 Feb. 2013, Orlando, FL.

Monitoring and Detection

- *Seasonal shifts in cLas prevalence in ACP.* Research was conducted in Florida from Jan 2008 to Feb 2012 from 6 sweet orange and 1 lime grove locations
  - cLas prevalence in ACP increased each year at all sites irrespective of management strategy
  - Female ACP cLas prevalence > male ACP
  - Brown-colored ACP cLas prevalence > green-colored ACP
  - Monthly variability
    - Oct through Dec had highest ACP prevalence of cLas

Ebert, TA, Brlansky, RH, and Rogers, ME. 2013. Seasonal shifts in cLas prevalence in the vector *Diaphorina citri* in Florida. IRCHLB Poster. 4-7 Feb. 2013, Orlando, FL.

Monitoring and Detection

- *Trap technology.* Chemosensory proteins are under study to identify those molecules that stimulate behavior responses in ACP
  - Commercial experimental product 'Titan' induced ACP probing/feeding behavior
    - When mixed with terpenes, these probing levels increased compared with the product alone
    - Male ACP probing behavior was comparable to that expected in the presence of orange jasmine flush
    - Female ACP behavior remained unchanged

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Patt, J. et al., 2013. a novel 'Arometic' compound acts synergistically with a naturally occurring monterpene to elicit strong behavioral responses in ACP. IRCHLB Poster. 4-7 Feb. 2013, Orlando, FL.

Monitoring and Detection

- MesaTech Molecular Diagnostic Platform
  - Handheld, self-contained, disposable
    - No external hardware requirements
    - Reagents enclosed within device
      - Plant tissue + buffer = test sample
    - Electrical adapter or battery powered (standard 9V battery)
  - Easy to use with a bacterial-based pathogen
    - Minimum user steps between sample and result
  - Sensitivity and specificity comparable to lab-based PCR
    - 2 temperature thermocycler amplification
  - Rapid: < 30 minutes sample-to-result

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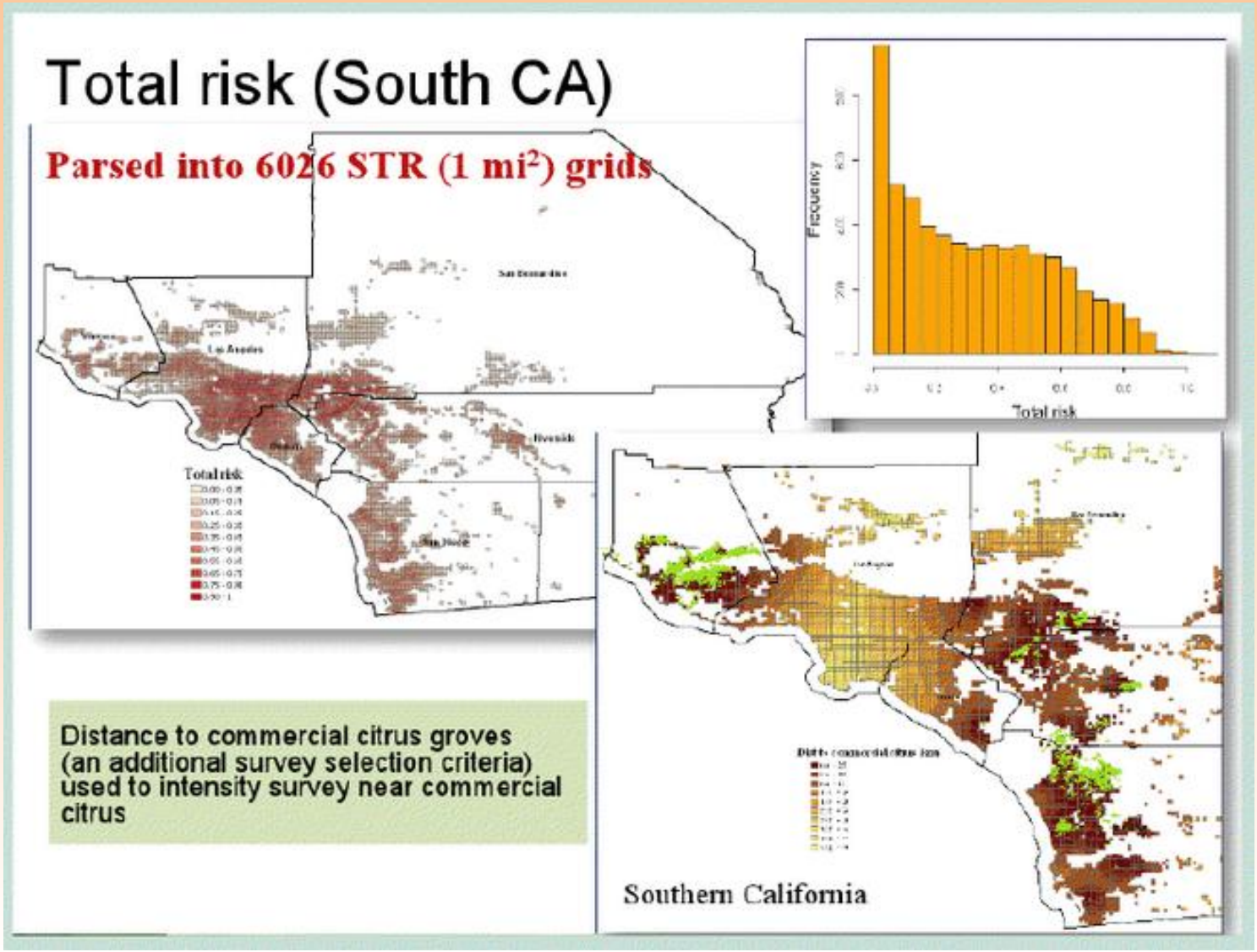
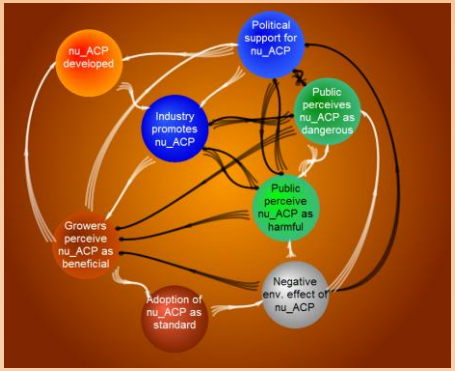
ACP/HLB risk modeling

Gottwald, Luo, McRoberts



nuPsylllid

(USDA CAP, Tom Turpen CRDF, PD)



Growers perceive nu_ACP as beneficial		0
Adoption of GM crops as standard		0
Negative env. Impact		1
Industry promotes nu_ACP technology		0
Nu_ACP developed		1
Political support for nu_ACP technology		0
Public perceives nu_ACP as dangerous		0
Public perceives nu_ACP crops as harmful		0

Growers perceive nu_ACP as beneficial		0
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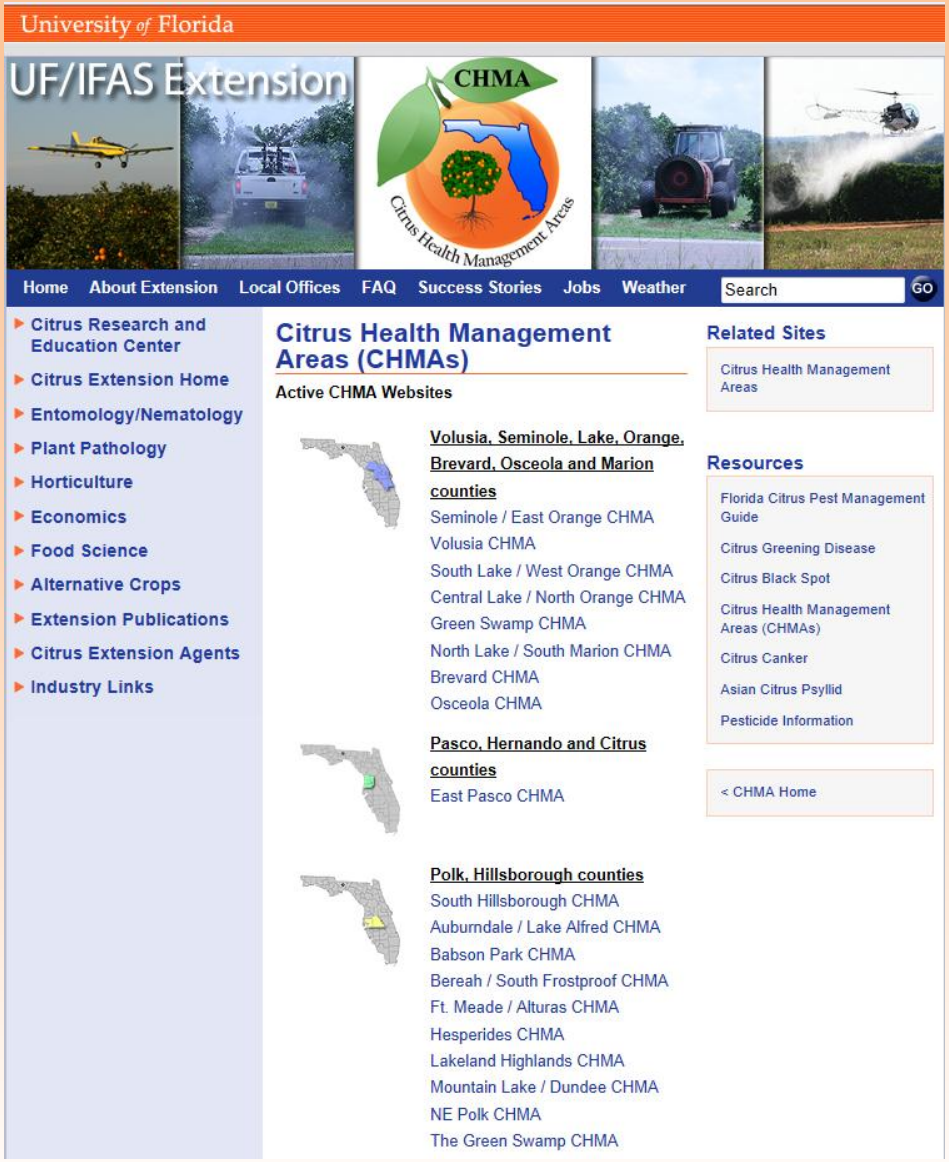
Mitigation and Disease Management

- *Plant thermotherapy to control disease.* Exposure to 20-42C for 7-10 days significantly reduced cLas titer in potted diseased citrus.
- cLas titers were significantly reduced
  - In dooryard trees
  - In commercial-use trees in the field, however titers increased again over time
  - Commercial citrus tented in May-June in Florida exhibited greater flushing with lower cLas titers

Doud, MS et al. 2013. Thermotherapy and chemotherapy to control citrus HLB in the field. IRCHLB Poster. 4-7 Feb. 2013, Orlando, FL.

Mitigation and Disease Management

- Citrus Health Mgmt. Areas
  - Area-wide control of ACP
    - Foliar application of broad-spectrum insecticides are applied during fall and spring to target over-wintering adult ACP
    - Applications are coordinated over a wide area



http://www.crec.ifas.ufl.edu/extension/chmas/index.shtml

Mitigation and Disease Management

- *RNA interference studies* passed to ACP by feeding indicate this technology can induce mortality in the absence of traditionally-known insecticide applications
- dsRNA added to artificial ACP diet at lower concentrations than previously published was effective at killing insects.

Ramos, J et al., 2013. RNA interference screening reveals redox processes to be responsive to low dsRNA doses in ACP. IRCHLB Poster. 4-7 Feb. 2013, Orlando, FL.

Liberibacter inoculation test on ‘Rio Red’ Grapefruit

Non-Transgenic buds grafted on Rootstock with infected buds

SoD2 Transgenic buds grafted on Rootstock with infected buds

Slide courtesy of Dr. T. Erik Mirkov, Texas A&M University

Mitigation and Disease Management

- Metalized mulch
  - significantly reduced ACP populations compared to a non-mulch control and a white-faced polyethylene mulch treatment
  - Increased soil moisture
  - Reduced weed pressure
  - Increased tree growth rate
  - Additional costs may be offset by increased tree vigor/production

Croxton, S. and Stansly, P. 2013. Metalized polyethylene mulch to reduce incidence of HLB and improve growth of new citrus plantings. IRCHLB Poster. 4-7 Feb. 2013, Orlando, FL.

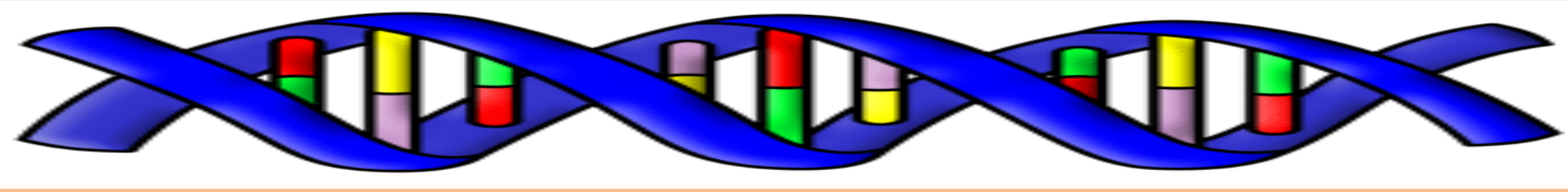
Genome Sequencing and Functional Genomics

Clas (China)

Claf (South Africa)

Clam (Brazil)

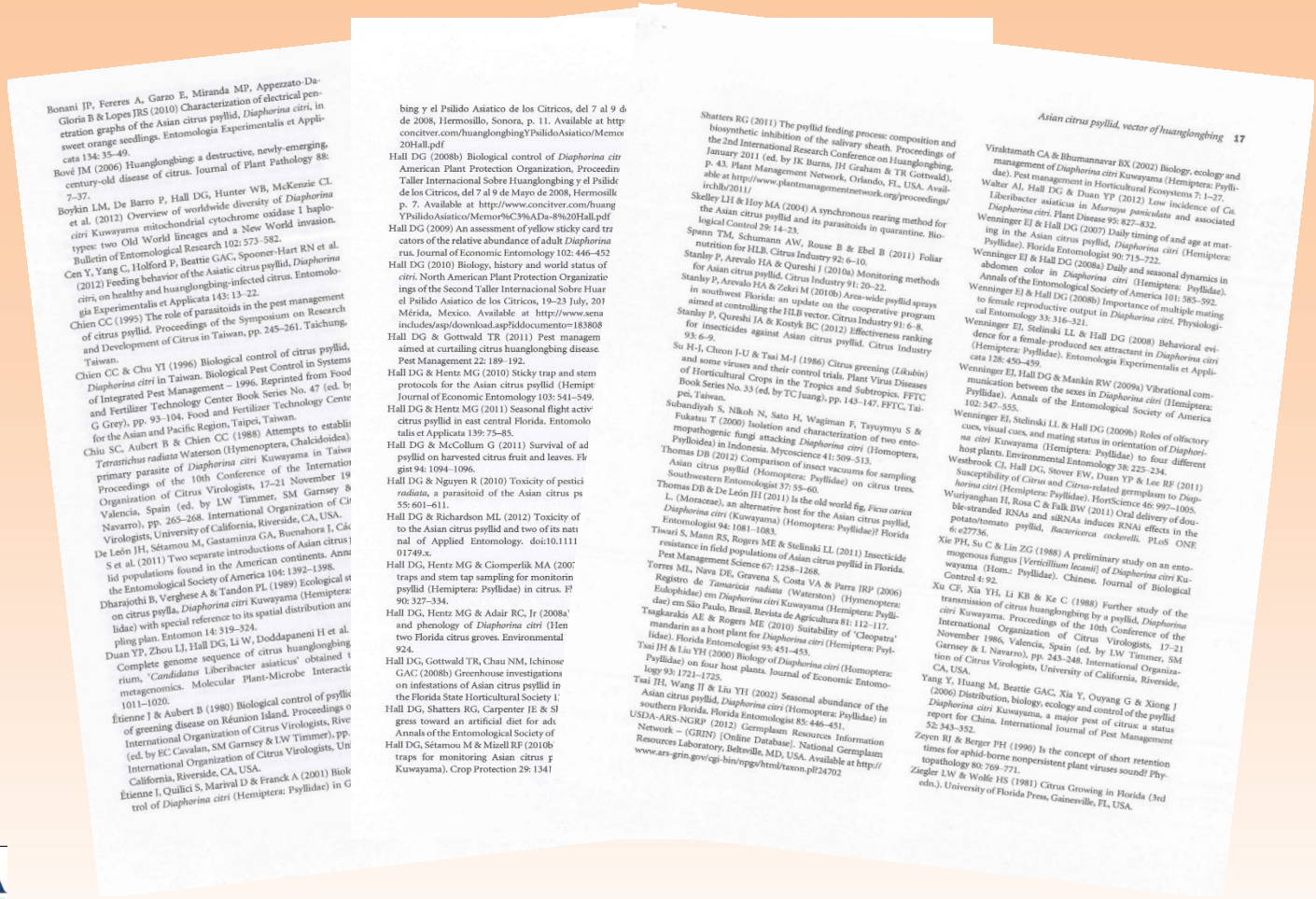
ACP



Mitigation and Disease Management

Flores, D. et al., 2013. Evaluating the biological control of ACP in the Rio Grande Valley of Texas. IRCHLB Poster. 4-7 Feb. 2013, Orlando, FL.

Peer-reviewed, Government, Extension, and Outreach Publications



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