INTERNATIONAL RESEARCH CONFERENCE ON HUANGLONGBING

Session 14b: Key Take-Home Messages abd a View to the Future: Summaries of Research Sessions

Orlando, Florida • December 2008

14.3 HLB and ACP situation and crop losses in Asia, South America and United States – T. Schubert

1) *Asia* - A. Beattie

Origin of ACP – India / Pakistan

- •Origin of Liberibacter –Africa (?) then to India
- •ACP spread from India / Pakistan to Taiwan and Indonesia in late 1800's, then through Asia in later decades
- •HLB spread from India / Pakistan to China about 1930, from China to Taiwan, Indonesia and Philippines in 1940's, more recently into Malaysia, Thailand and southern islands of Japan
- •HLB generally present throughout Asia, absent from Japan mainland but moving North
- •HLB absent in Three Gorges area of China, geographic and mountain separation

Production decreasing on per ha basis

- •Much ineffective pesticide use; oils perform well, help slow infection rate
- •Clean nursery stock becoming available but costly, demand exceeds supply
- •Little coordination or uniformity of area-wide management or education efforts
- •Infection is early, rates high
- •Prognosis poor for foreseeable future
- •Yields and $ returns too low to afford effective management
- •Some use of guava interplanting in Vietnam

***2) Brazil* - C. Montiero**

ACP first detected in 1942

- •HLB first detected in 2004
- •3^{rd} species – *L. americanus*, 2005
- •Now L. asiaticus predominates

L. americanus only known in Brazil at present

- •Other citrus in South America at great risk
- •In 2008, 41% of all citrus is in a block with at least one HLB infected plant
- •< 28% detection of HLB →rogue infected only
- •>28% detection of HLB →block removal

HLB incidence in Brazil

In 2004, 3 states, 3.4% average incidence in 46 municipalities of Sáo Paulo state only; In 2008, 18.6% average incidence in Sáo Paulo state (0.7-27.5% in 5 management areas)

Brazil Crop Losses –

- •Over 1,000,000 trees removed to date
- •Compulsory management enforced

No data on decline in juice quality

- •Yield reductions related to HLB severity by negative exponential model
- •Yield reduction due to fruit drop and failure to set new fruit
- •Overall production down from previous highs, but other factors in addition to HLB
- •By 2020, production predicted to be down 25% due to HLB

3) ***United States*** **- J. Snively**

Florida - ACP arrived in 1998, HLB in 2005

- •HLB incidence expanded from 2 to 32 counties in 3 years
- •Worst blocks up to 80% symptomatic
- •ACP has spread west, TX in 2001
- •Will reach extreme southern CA by 2008 coming from Mexico
- •Only HLB known to exist outside of FL is small amount in southern LA

US Crop Losses

Little to no impact on juice quality noted so far

Quality of juice from symptomatic fruit rated lower that asymptomatic fruit from symptomatic tree which tastes the same as fruit from healthy tree

Symptomatic fruit can be graded out by size

Overall FL production down from previous highs, but other factors in addition to HLB

By 2020, production predicted to be down by 15-20% due to HLB

HLB management increases costs $474/A, up 38%

4) ***Conclusions***

ACP precedes HLB by few to several years

- •Infected plants probably precede psyllids, but disease is stationary and a dead end until vector goes to work
- •Latent period variable: months to 3-5 yrs
- •Profitability depends on comprehensive management which depends on crop value
- •Growers not easily convinced management is worth the cost, many complex factors to consider
- •Need area-wide management approach to counteract bad neighbor effect – How?

14.4 HLB Survey – M. Irey

1) Data trends from an HLB laboratory in a commercial lab open to the industry
- •Make the "right thing easy" for the growers
- •Get growers "on a program"
- •~65,000 samples processed over a 2-year period
- •Sample volume increasing
  - –More disease
  - –More awareness
- • Data Trends associated with samples
  - –Best time for symptoms –Late Jul to Mar
  - –Highest bacterial titer –July to Feb
  - –Most susceptible –trees 6-9 yrs old and 6-9 ft tall
  - –Most susceptible - grapefruit and round oranges
  - –Least susceptible – tangelos and tangerines
  - –More infection on edges

2) Texas surveys for HLB and ACP
- •ACP present in TX, HLB not detected
- •After HLB scare based on psyllid testing, survey efforts have been stepped up
  - –Commercial industry in south Texas
  - –Residential and specialty plantings throughout state
- •APHIS certified lab
  - –822 plant samples to date
  - –815 psyllids to date
  - –All negative
- •Working to enclose budwood sources
- •Have multi-language extension efforts
- •In process of developing state action plan
- •Area-wide psyllid control pilot plan
- •Continuing APHIS supported survey

3) National plan for the detection of HLB in Mexico
- •Psyllids found in 2002, now present in all states with commercial citrus
- •Implementing an early detection program to detect HLB
  - –Surveys
  - –Vector testing
- •Amount of testing based on 3 defined risk categories which increases with risk category
- •Have authorized HLB laboratories
  - –Pictures of suspect samples sent to central source
  - –Based on photos, decision to sample or not is made
- •State personnel have been trained to detect HLB in Florida and by in-house training
- •Testing to date
  - –155 psyllid
  - –26 plant samples
  - –All negative

4) Survey for Candidatus Liberibacter species in South Africa
- •Ca. Liberibacter africanus and Trioza in South Africa many years
- •No recent surveys in SA using modern PCR techniques
- •2006 – 249 samples from 57 orchards
  - –Only *Ca.* L. africanus found on citrus in SA
- •No strain variation in *Ca.* L. africanus
- •Also sampled indigenous Rutaceous plants
  - –Only found *Ca.* L. africanus capensis
- •Fair amount of variability in isolates and more widespread than expected

5) Movement of HLB in Florida
- •ACP in Florida 1998
- •ACP largely spread throughout state by *Murraya paniculata*
  - –Initial population probably free of HLB
- •Spread of HLB
  - –Estimated flight of ACP 1.5 km
  - –Spread of HLB too fast to be due solely to flight of ACP
  - –Have found positive ACP from nursery plants (Citrus and Murraya)
  - –Estimate that ACP can travel 54 km
- •Murraya played a significant role in distribution of ACP and HLB in Florida
- •Testing of psyllids is a valuable tool for early monitoring of HLB

6) Is it possible to replant young groves in an area with endemic HLB?
- •Hierarchical approach is needed because it isn't possible to sample all trees
- •Management decision: Should we and can we replant in the presence of HLB?
- •Following 2 blocks (~30,400 trees)
  - –Aggressive insect control
  - –6X scouting/yr
  - –PCR testing
- •Results to date 0.4% after 2.5 yrs

14.5 Liberibacter sequence characterization and culture – W. Dawson

1) Candidatus Liberibacter americanus, associated with citrus HLB in Brazil has three ribosomal RNA operons and a genome size of 1.34Mbp
2) Features of '*Candidatus* Liberibacter asiaticus' genome

   Ca. Liberibacter asiaticus (Las) draft genome has 1124 annotated proteins
   Ca. Liberibacter solanacearum (Lso) draft genome has 1128 annotated proteins
   Common and unique proteins between citrus and potato Liberibacters:
   common 815, Las 209 unique, Lso 211 unique

   3) Evaluation of potential pathogenicity genes identified by genomic sequencing of *Ca*. Liberibacter asiaticus – D. Gabriel et al.

   Type I secretion system

   - Secretion of proteins produced by the bacterium
   - Efflux of toxic substances from the bacterium

4) Several Liberibacter and Phytoplasma Species are Individually Associated with HLB: Towards a common denominator.

   The different bacteria associated with HLB-like symptoms are all sieve tube-restricted.
   They might have similar pathogenicity mechanisms resulting in similar disease symptoms. However, not all sieve tube-restricted bacteria have similar pathogenicity mechanisms, and a given bacterium might have several such mechanisms.
   Experiments to check the hypothesis are underway.

5) Zebra Chip Complex and HLB Disease symptom is discoloration of potato tubers resulting in lot rejection

   Pathogen: *Candidatus* Liberibacter psyllarous
   Insect Vector: The Potato Psyllid (other psyllids?)
   Are They Potato Psyllids?

   •Over 70 genera of psyllids are grouped under family Psyllidae
   •The COI sequence has been used to identify psyllids.
   •Hackberry psyllid (*Pachypsylla* sp.) and mesquite psyllid (*Heteropsylla texana*) COI sequences from South Untied States and Mexico were published.

6) Cocultivation of *Candidatus* liberibacter asisaticus with actinobacter from Citrus with HLB
7) Development of an Asian citrus psyllid (*Diaphorina citri*) insect cell line

14.6 Host-Pathogen Interaction – J. daGraça

1) Seed transmission findings unresolved
   Seed from 8 spp.; >1250 tests by qPCR–no positives New assay gives positives but low titer; 20/69 sour orange with Ct from 32-39
   Transient detection in Carrizo citrange seed
2) Sequence of anatomical events
   •Infection – long incubation period of build-up to a critical titer
   •Phloem plugging – 2 types, phloem necrosis follows (toxin or starvation?)
   •Sugar transport blocked > starch accumulation
   •Loss of internal structure of chloroplasts
   •Chlorosis of leaf areas distal to blocked phloem producing mottled chlorotic pattern
3) Host molecular responses
   •Microarrays –> 600 genes up and down regulated
   •Included defense regulators, phytohormones
   •3 genes in starch synthesis up-regulated
   •Phloem proteins up-regulated
      Possibility to suppress the HLB symptoms by controlling the expression of genes encoding callose and phloem proteins?
      Investigation needed for blockage of the phloem, effects on loading of nutrients, and nutrient transport.
4) Rootstock effects on HLB?
   Feroniella rootstock does not confer HLB resistance to the scion
5) Solanaceous Liberibacter is a new *Ca*. Lib. spp. in tomato and potato + psyllid vector. May be useful as a model systems for citrus liberibacters – Liberibacter asiaticus infects tomato, but solanaceous liberibacter was not found in citrus or D. citri.
6) Titer Las > Lam; greater temp. sensitivity of Lam in Murraya; Lam disappearing in citrus in São Paulo State Brazil
7) Different strains of Las in Japan due to mutations or new introductions? Pummelo was resistant but now is no longer resistant. Is host range of new strains expanding?

14.7 Asian Citrus Psyllid (Biology and Genomics) – D. Hall

1) Gene expression in midgut tissues of *Diaphorina citri*: application to biology and vector control

RNA inhibition strategies to control the psyllid and spread of HLB involving tissue-specific gene silencing through RNA interference (i.e. RNAi control).

The sequencing data includes midgut and testes tissues.

2) Pheromones of the ACP elicit behavioral responses from its parasitoid, *Tamarixia radiata*

- Isolation and identification of semiochemicals associated with the parasitoid *Tamarixia* and its host *Diaphorina*.
- Male and female *Tamarixia* apparently produce some of the same pheromone components that the psyllid produces.
- Male Diaphorina and both sexes of *Tamarixia* showed attraction to synthetic γ-Butyrolactone
- Female *Tamarixia* probably cue on volatile compounds produced by *Diaphorina* for host location.
- Research in this area could be fruitful with respect to enhancing biological control of the psyllid, particularly in urban settings and abandoned groves.

Attractants such as γ –Butyrolactone might have applied applications for psyllid control.

3) Effects of freezes on survival of *Diaphorina citri*.

- The research presented indicated that some freeze events particularly in northern areas of Florida might be severe enough to temporarily eradicate the psyllid.
- The research provided some insight into where across the USA the psyllid might or might not be able to survive during the winter.

4) Characterization of electrical penetration graphs of *Diaphorina citri* in citrus

Information on feeding activities by the psyllid that may be applied with respect to understanding transmission of the HLB pathogen.

5) Symbionts associated with ACP in Brazil and a look into their role

- Symbionts play a major role in determining ACP fitness.
- ACP in Brazil harbors four different symbionts: *Carsonella, Wolbachia, Syncitium* and *Pantoea.*
- Antibiotic treatments reduced levels of Wolbachiain psyllids, with corresponding increases in levels of the other three symbionts.
- Reductions in *Wolbachia* resulted in reductions in egg and nymph survivorship, although the effect on egg survivorship seems to be related to an undesirable effect of the antibiotic treatment itself rather than reduced levels of the symbiont.
- Novel management tactics for the psyllid might be developed by manipulating symbionts associated with ACP.

6) Endosymbiotic microbiota of ACP

- Psyllids feed from the phloem of plants ingesting a diet that is rich in carbohydrates but deficient in essential amino acids. Endosymbionts support the psyllid by supplying essential amino acids.
- Knowledge of the bacterial community of psyllids could provide information relevant to future psyllid management strategies through manipulations of its endosymbionts.
- Seven different endosymbionts of the psyllid were reported.
- Many functional bacterial homologies were isolated within the psyllid.

- •Interactions between psyllid endosymbionts have important ramifications not only on psyllid biology but also on the endosymbionts themselves and with other bacteria such as Liberibacter asiaticus when introduced to the psyllid.

7) FK506-Binding protein from ACP
- •Binding proteins in insects have been shown to play a role in calcium regulation.
- •A binding protein associated with ACP was isolated and characterized.
- •This binding protein may be a good genetic target for an RNA inhibition approach to managing the psyllid.

8) Gene expression in ACP adults feeding from Florida citrus: Application to biology and vector control
- •Genomics approach to study the genetic basis of the biology of *D. citri* and identified a number of specific genes associated with feeding, reproduction, and insecticide resistance.
- •These genes could be targets for RNA inhibition tactics aimed at controlling the psyllid and reducing the spread of citrus greening.

9) Asian citrus psyllid, genetic basis of immunity
- •ACP gene responses to imidacloprid and other stress factors such as temperature.
- •The genes could be targets for RNA inhibition tactics aimed at controlling the psyllid and reducing the spread of citrus greening.

10) Effects of host plant on fitness of ACP
- •Comparisons of psyllid fitness on two rootstocks, sour orange (*Citrus aurantium*) and Cleopatra mandarin (*Citrus reticulata*)
- •Clear evidence that Cleopatra mandarin is an unsuitable host plant for ACP.

11) Development of a potato psyllid (*Bactericera cockerelli*) cell culture
- •Development of a potato psyllid cell culture which is expected to allow researchers a new approach for rearing Liberibacter associated with potato and to screen peptides and other chemicals for toxicity to the potato psyllid.

14.8 Psyllid Management Strategies – M. Rogers

1) Biology and ecology of *Diaphorina citri* and *Tamarixia radiata* in São Paulo State.
- •Determine optimum conditions for D. citri and T. radiata development for evaluation of the effectiveness of the parasitoid
- •Ecological zoning for D. citri and T. radiata based on temperature requirements.
- •To provide basic information for D. citri to facilitate detection, monitoring, and control.

2) Evaluation of the Efficacy of Guava against ACP
- •A few years ago Vietnamese farmers noticed that the citrus psyllid population and HLB incidence were low in citrus groves inter-planted with guava trees. The observations were confirmed by researchers at the Southern Fruit Research Institute.
- •This discovery may lead to new approaches for the environmental friendly control measures, i.e. inter-planting or use of active compounds from guava.
- •For purpose of understanding what caused the reduction of psyllid and HLB incidence in the inter-planted citrus groves, experiment were designed.

3) Wounding of Guava leaves produces defensive sulfur compounds.
- •Identify the active components (volatiles) in guava leaves that repel or reduce psyllid populations and avoid the negative aspects of guava trees/fruit.
- •Guava leaves produce Dimethyl disulfide (DMDS) when mechanically injured. DMDS is not produced by citrus. DMDS is highly toxic to most insect species and is one possible explanation for the repulsive effect of guava on the ACP.

4) Chemical ecology of ACP and potential applications of behavior-modifying chemicals for its management.
- •Investigating the ACP antenna, the sensilla and their probable functions
- •Chemical communication between male and female ACP-towards identification of a sex-attractant pheromone
- •Responses to host plant volatiles
- •Development of a Guava-based repellent
- •Analysis of ACP head-space volatiles
- •84 compounds identified: 7 Male-specific,14 Female-specific, 40 Shared, 12 induced compounds (physically isolated, but chemically interacting), 7 induced compounds (physically & chemically interacting)
- •Behavioral responses of male and female ACP to γ-Butyrolactone – male specific
- • Investigating potential role of Guava as an ACP repellant

5) Novel reovirus in ACP
- •Localization of reovirus in psyllid – present in all stages or tissues except eggs.
- •Population of reovirus in Ft. Pierce, FL
- •Psyllids which were collected from the field (May 2008) resulted in ~55% virus positive.
- •No immediate pathogenic effects on ACP were observed.
- •Virus acquisition and transmission may be occurring due to a combination of the ACP's feeding behavior and wide citrus host range which may act as reovirus host plants.

6) Efficiency of insecticides for control of ACP
- •Efficiency of systemic insecticides applied in the nursery to control ACP
- •Determine the residual period for insecticides.

- •Soil applied insecticides in nurseries, before planting are most the effective method for control of ACP in the field (imidacloprid ~ 70 days; thiamethoxam and clothianidin 70-105 days; acetamiprid ineffective)
- •Foliar applied insecticides that provided the longest residual period are: thiamethoxam, imidacloprid, formetanate, thiamethoxam + lambda-cyhalothrin, dinotefuran, gamma-cyhalothrin, methidathion and fosmet (effective for ~ 34 days after application)

7) Integrated management of ACP in Florida

•Suppression of psyllid populations by predacious insects.

•Incidence of ACP parasitism by *Tamarixia radiata* is highest in Central FL

Diaphorencyrtus aligarhensis (Encyrtidae) collected in Guangdong China Sep 2006

•Endoparasitoid attacking 2nd – 4th instars

•Host feeds on 1st – 4th instar nymphs.

•Currently being released in Florida

Tamarixia radiata

•Colonies from new collections made in Pakistan, Vietnam and Gongdong China awaiting in quarantine at FDACS-DPI for USDA-APHIS release permits.

Psyllid management plan for mature citrus

•Monitor populations year round

•Dormant spray(s) with short PHI adulticide

•No sprays on flushes

•Only soft chemistry post bloom

•Oil program option throughout growing season

•OPs in summer and before fall flush if necessary

14.9 HLB Management – A. Beattie

Juliano Ayres, presenting for J Belasque et al.

Noted critical practices were required for 'control' (effective management) of huanglongbing (HLB) in São Paulo:

1) eradication of diseased trees (detected by scouting, confirmed by PCR
2) 'control' (suppression of *Diaphorina citri*) with insecticides,
3) and use of pathogen-free trees.

4) He added that 8 factors were associated with HLB 'control' in Sao Paulo,:
    a. farm location
    b. distance from 'bad neighbour'
    c. farm size average
    d. age of trees
    e. timing of eradication,
    f. number of sprays per yearn
    g. umber of inspections per year
    h. accummulated HLB
    i. incidence in first year.
2) He gave examples illustrating the importance of these points: the key to survival being no bad neighbours, eradication, inspections, sprays.
3) The message was –'if you start late you will lose the war'.

Manjunath Keremane, on behalf of Manjunath et al.

Noted the importance of testing sampled psyllids, particularly nymphs, as a means of early detection of the possible presence in blocks of asymptomatic orchards –possibly 3 months before infected trees develop symptoms.

He also highlighted the need to sample from various locations –including backyards and retail outlets.

Mike Irey, on behalf of Irey et al., spoke on:

Scouting

Tagging

PCR

Tree eradication at Southern Gardens

Edge effects and invasions of D. citri and, axiomatically, HLB from 'commencing' in the east and southeast corners of blocks, and hot-spots associated with breaks or 'interfaces' in blocks (e.g., ponds). These were interesting observations.

In the reply to a question on 'resets', he considered (a) such practices unwise and expressed the opinion that the future of the Florida citrus industry may depend on rotational blocks.

Roberto Bassanezi, on behalf of Bassanezi et al.

Noted that HLB management required continuous and costly efforts.

He summarised two large scale factorial experiments, albeit with replicates comprising 504 to 528 trees.

The first, in an block planted in 2005, compared scouting and tree eradication (28, 56 and 112 days) and psyllid control (no sprays, sprays every 14 days, and sprays every 28 days).

The orchard was 1.8 km from its nearest neighbour.

The insecticides were aldicarb and thiamethoxam to soil in the rainy season at 56 day intervals and imidacloprid, dimethoate and lambda-cyhalothrin in the dry season every 14 or 28 days.

In September 2008 (36 months), cumulative numbers of psyllids caught in traps and HLB incidence.

Experiment 2 was established in a small farm without citrus groves, but until the beginning of 2007 it was surrounded by severe HLB-affected farms.

It compared scouting and tree removal every 14 days, 28 days, 84 days and 182 day) and four psyllid treatments, no psyllid control and sprays applied every 14 days with lambda-cyhalothrin replaced with etofenprox. After 29 months there were some significant differences between cumulative numbers of psyllids in the controls and insecticide treatments but none fro HLB levels.

Katsuya Ichinose, on behalf of Ichinose et al.

Presented results of trials comparing systemic insecticides (alone) and guava (interplants alone).

Levels of HLB rose relatively quickly in all treatments, and the results cast doubt on the efficacy of guava interplants.

Jim Graham, on behalf of Graham et al.

Presented results on the potential use of imidacloprid to induce systemic acquired resistance to reduce levels of HLB in Cleopatra mandarin.

These results did not demonstrate a reduction in symptom expression and bacterial titre in imidacloprid-treated plants.

This may have been due in part to high-inoculum potential in the experiment, which was undertaken in a glasshouse.

Field pressure may have been lower.

14.10 Host Resistance - E. Stover

Objectives

- Explore resistance to HLB in existing citrus varieties or citrus relatives
- Identify transgenes that may provide resistance to HLB and other diseases
- Develop and test promoters and regulatory elements to control expression of resistance genes
- Produce and test transgenic citrus with potential resistance

1) Explored resistance to HLB in existing citrus varieties or citrus relatives in Brazil
   - Evaluated HLB and Las progression after graft inoculation of 6 genera related to citrus.
   - Atalantia, Eremolemon and Poncirus showed slower development of Las
   - Only Atalantia had some replicates that were PCR- after 6 months

2) Evaluated psyllid colonization in numerous Citrus species and genera related to citrus in Indonesia
   - Psyllids heavily colonized Bergera, Murraya, Swinglea, citron, and C. hystrix, with few on other genotypes, including mandarins and pummelos
   - After two years, few plants were PCR positive for Las

3) Screened 31 Citrus varieties and relatives in Florida.
   - In tolerant plants tested, incubation under continuous light produced symptoms. Could this also happen under other stresses?

4) Tested diverse citrus in Thailand for HLB resistance and found all to be susceptible.

5) Explored HLB and Las in 13 selections of local mandarin in Japan.
   - 12 developed distinct symptoms of HLB, one did not

   Seedlings of Unzoki grew as vigorously as healthy ones 11 months after graft-inoculation, but tested positive for Las in PCR.

6) Resistance of somaclonal variants or varieties to CVC - possible extension to HLB?

7) Conclusions:
   - So far, exploration of existing resistance in citrus and relatives appears to reveal tolerance but not resistance
   - Could be basis for future industry, but would provide "typhoid Marys" alongside existing trees

8) Identifying genes for transgenic HLB resistant citrus
   - Transgenics appear to be the only medium term solution for HLB resistant citrus
   - genes which should contribute to broad resistance are needed, too little is known about host / pathogen interaction for HLB-specific target
   - antimicrobial peptides are major focus
   - some groups have focused on enhancing SAR
   - citrus genes associated with resistance for Cis-transgenics
   - Virus genes which may disrupt pathogen functions

9) Antimicrobial peptides (AMPs)?
   - Antimicrobial peptides are small proteins, usually 12 and 50 amino acids.
   - They form the first line of host defense against pathogenic infections and are a key component of the innate immune system
   - Antimicrobial peptides are involved in the antimicrobial defense system among all classes of life: plants, insects, amphibians and mammals including humans

   Selection of AMPs

- •Plant-derived or synthetic for greater consumer acceptance
- •Low potential for adverse health effects
- •Reports of effectiveness against related bacteria

Several Antimicrobial gene(s) currently under evaluation at CREC breeding program

- •Attacin E-Lytic peptide gene from Hyalophora cecropia.
- •CEAD-Codon optimized cecropin A-cecropin D lytic peptide gene.
- •CEMA-Codon optimized cecropin A-melittin lytic peptide gene.
- •CEME-Codon optimized cecropin A-melittin lytic peptide gene (differs at the *C terminus* from CEMA).
- •LIMA-Lytic peptide gene.
- •PTA-Codon optimized N terminally modified Temporin A gene.

In –Vitro AMP Screening USHRL

- •Agrobacterium and Sinorhizobium are related to Liberibacter
- •Also using Xanthomonas
- •Best AMPs, including D4E1 are effective in 1 μM range

10) Genes for SAR at CREC and Gainesville

- •NPR1 (Nonexpresser of PR Genes - 1 gene from Arabidopsis) – NPR1 is a key regulator in the signal transduction pathway that leads to SAR.
- •SABP2 (Salicylic Acid-Binding Protein 2 gene from tobacco) – high affinity for SA.

11) Identifying bacteriophage genes to kill the pathogen

- •Isolated bacteriophage and sequenced to identify diffusible killing factors
- •Transformed into model plant systems and identified some promising results with model systems

12) Allelyx Strategies

- •Defense responses
    - •TF: transcription factors
    - •AMP: antimicrobial peptides from citrus species
    - •28 candidate genes, more than 1000 transgenic plants

13) Sequencing of Liberibacter

- •Availability of sequence data will open up new opportunities
- •Should permit identification of genes specific to pathogenicity
- •Allow targeting of HLB-specific solutions through transgenics

14) Developing and testing promoters and regulatory elements

Use of phloem specific promoters to restrict trans-protein in phloem tissues

- •HLB resides in the phloem
- •Several groups are comparing universal promoters, which may also permit control of canker etc vs. phloem specific which may be more effective against HLB and may reduce transgene in fruit

15) Transgenic efforts on developing HLB-and canker resistant citrus

- •Transgenics appear to be the only medium term solution for HLB resistant citrus
- •Using genes to disrupt the pathogen or the vector
- •With so little known about host / pathogen interaction, genes with effects on many bacterial pathogens are needed
- •Antimicrobial peptides is a major focus
- •Also focused on enhancing SAR
- •One has identified citrus genes associated with resistance for Cis-transgenics

16) Transgenic Projects:

- •Some but not all of the transgenic strategies are looking very promising
- •Several groups are moving ahead fast to produce resistant plants with the best available technology
- •At the same time, they and other labs are working to identify other genes/promoters etc.
- •which may be more effective
- •and may be more easily accepted by regulators and consumers