

# **2nd International Citrus Canker and Huanglongbing Research Workshop**

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## **Huanglongbing**

- **Research Recommendations – HLB**
- **HLB Priority Research Recommendation Chart 2005**
- **HLB Priority Prioritized Research Recommendation List**

## **Citrus Canker**

- **Research Recommendations – Citrus Canker**
- **Citrus Canker Priority Research Recommendation Chart 2005**
- **Citrus Canker Prioritized Research Recommendation List**

**Presentation on following pages...**

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- Economics
- Alternate Hosts
- Detection of Disease/Vector
- Differentiation
- Resistance and Breeding
- Culturing HLB
- Pathogen/Vector Interactions
- Citrus Genetics
- Chemical Control
- Biological Control
- Cultural Control
- Pathogenesis
- Epidemiology
- Transgenics
- Genomics
- Vector Biology
- Fruit Yield/Quality

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□ **1 Economics**

■ 1.1 Economic Analysis

- Economic losses due to restricted movement
  - Economic analysis of lost markets (domestic and international)
  - Economic benefit of tree removal
  - Economic analysis of control measures
  - Phytosanitary systems for fruit movement from quarantine areas
  - Rotational/cropping systems
  - Ornamental industry
  - Pest risk analyses
  - Phytosanitary measures for nurseries
-

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□ **2 Alternative Hosts**

- 2.1 Identify alternative hosts of pathogen/vector and geographic distribution
  - 2.2 Bacterial population level in alternative hosts
  - 2.3 Risk assessment of alternative hosts
  - 2.4 Vector biology ref. alternative hosts
-

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□ 3 Detection of Disease and Vector

- 3.1 Field vs Nursery Symptomology
  - 3.2 Development Of New Serological Tools
  - 3.3 EM, Light Microscopy, CF
  - 3.4 Molecular Detection Methods
    - Dot blot probes (vectors)
    - PCR – 16S RNA; rpl-based PCR; Duplex: nested PCR; Multiplex Real-time PCR
    - Improve detection (sensitivity); understand host-pathogen interaction leading to better control, etc.
  - 3.5 Sentinel Indicator Plant
  - 3.6 Sample Criteria
  - 3.7 Psyllid Population Density Sampling
  - 3.8 Chemical or Volatile Detection
  - 3.9 Storage of Samples Effects
  - 3.10 Remote Sensing
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- **4 Characterization Taxonomy of HLB**
    - 4.1 Differentiation of species and strains
    - 4.2 Genetic diversity studies
    - 4.3 Repositories for voucher specimens
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□ **5 Resistance and Breeding**

- 5.1 Assessment of Citrus Relatives For Resistance/Susceptibility
  - 5.2 Cold Hardiness for Citrus
  - 5.3 Host Genotype Strain/Species Interactions
  - 5.4 Vector Repellency/Lethal Genes
  - 5.5 Field Testing of Cultivars
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- **6 Culturing HLB**
  - 6.1 Culturing HLB

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- **7 Pathogen/Vector/Host Interactions**
    - 7.1 Population In Different Cultivars
    - 7.2 Pathogen Vector Relationships
      - Transovarial Transmission
-

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8 Chemical Control

- 8.1 Insecticides To Keep Psyllids Off Nursery And Grove Bearing And Non-bearing Trees
    - Scouts
    - Encourage Beneficials
  - 8.2 Effect of Insecticides on Disease Spread
    - Duration of Protection
  - 8.3 Urban Homeowner Control of Psyllids
  - 8.4 Systemic Bactericide
  - 8.5 Baseline Toxicity Studies
  - 8.6 Natural Chemicals
  - 8.7 Application Methods and Interaction With Beneficials
  - 8.8 Chemicals to Control Flush
  - 8.9 Effect of Toxicants On Vector Transmission
-

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□ **9 Biological Control**

- 9.1 Determination of Presence of Hyperparasites
  - 9.2 Foreign Exploration
  - 9.3 Trap Plants (Mp Et Al)
  - 9.4 Biological Control of Pathogen
  - 9.5 BC of Vector in Residential Areas
  - 9.6 Distribution of Parasitoids and Efficiency
-

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□ **10 Cultural Control**

- 10.1 HLB-free budwood
  - 10.2 Nursery design, management and location away
    - Budwood sources/nurseries under screen
  - 10.3 Pruning and rogueing
  - 10.4 Orchard design and management
    - Intercropping
  - 10.5 Greenhouse production of citrus
  - 10.6 Cultural control of alternate hosts
-

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□ **11 Epidemiology**

- 11.1 Invasive Potential of Disease And Vector
  - 11.2 Effect of Cultural Practices
    - Effect Of Effect of Vector Control on Disease Development
    - Rogueing
    - Effect of Trap Plants
  - 11.3 Effect of Insect Population Dynamics on Disease Dynamics
  - 11.4 Distance of Disease and Vector Spread
  - 11.5 Aging Infection
  - 11.6 Seed Transmission and Graft Transmission from Asymptomatic Plants
  - 11.7 Proportion of Infected Insects in Population Relative to Disease Incidence
  - 11.8 Survey of Incidence and Distribution in FL
  - 11.9 Eradication Methods
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## □ 12 Transgenics

- 12.1 Find and introduce resistance:
  - 12.2 Resistance genes in related spp.
    - Anti-bacterial genes
    - Anti-bacterial peptides
    - Use of viral vectors
    - Anti-insect genes
  - 12.3 Rapid screening method for resistance
  - 12.4 Genetic modification of vector
  - 12.5 Development of transformation methodologies for citrus and citrus relatives and ornamentals
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□ **13 Genomics**

- 13.1 Citrus Responsive Genes for Early Detection
  - 13.2 Sequencing of Bacterial Genomes
  - 13.3 Sequencing of Citrus Genome
  - 13.4 EST Microarray
  - 13.5 Comparative and Functional Genomics
-

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□ **14 Vector Biology**

- 14.1 Reproductive Biology And Behavior
  - 14.2 Pheromones and Attractants
  - 14.3 Dispersal Behavior of Vector
-

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■ **15 Fruit Yield and Quality**

- 15.1 Relationship of Fruit Quality to Disease Incidence
  - 15.2 Physical Means for Culling
  - 15.3 Crop or Yield Loss Models
-

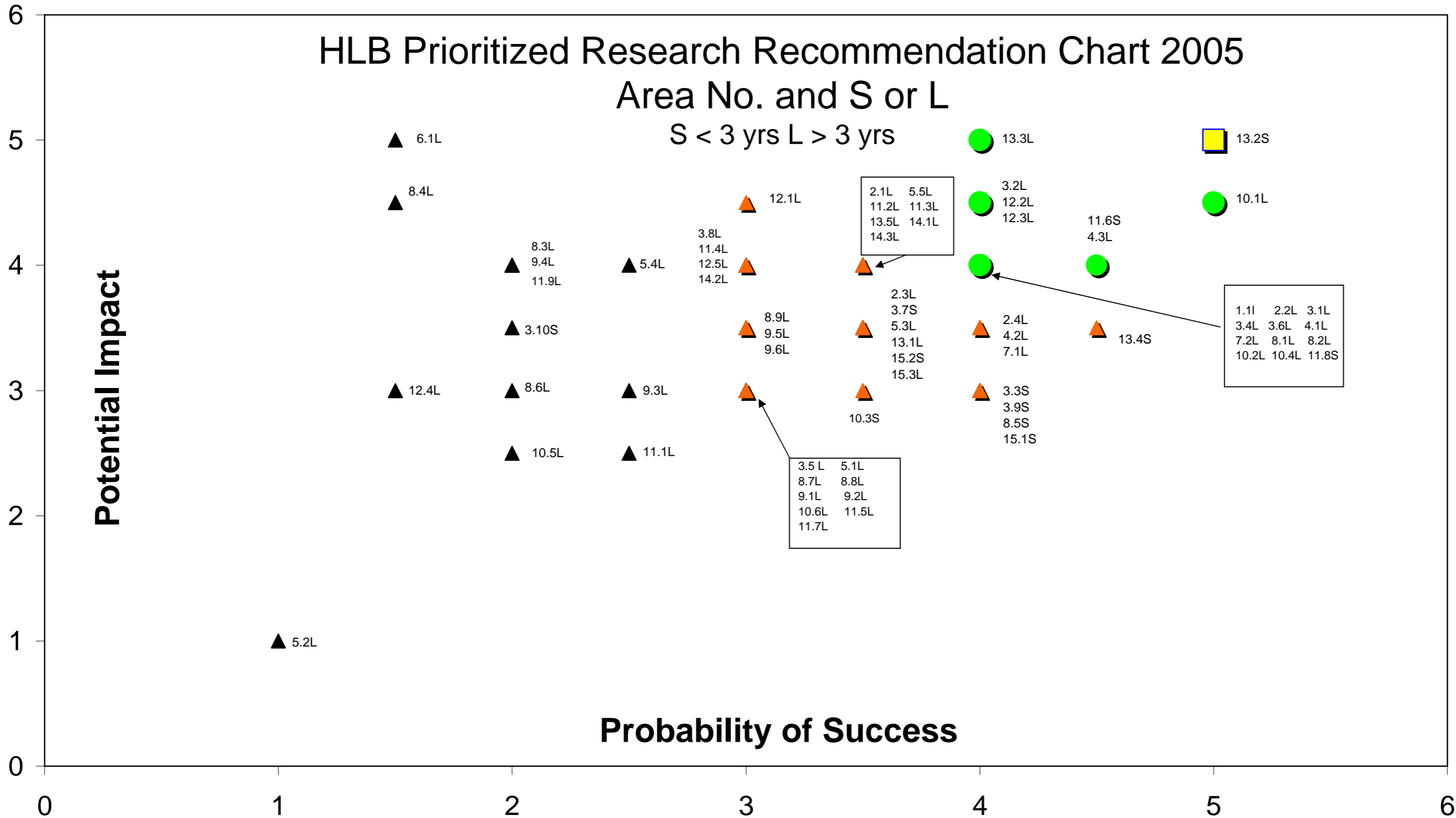
# HLB Prioritized Research Recommendation Chart 2005

## Area No. and S or L

S < 3 yrs L > 3 yrs

Potential Impact

Probability of Success



## Huanglongbing Prioritized Research Recommendation List

Scale is 1 (low) to 5 (high)

Area No.	Project	Short Term < 3yrs	Long Term > 3 yrs	Probability of Success	Potential Impact
1	<b>Economics</b>				
	1.1		x	4	4
2	<b>Alternative Hosts</b>				
	2.1		x	3.5	4
	2.2		x	4	4
	2.3		x	3.5	3.5
	2.4		x	4	3.5
3	<b>Detection of Disease and Vector</b>				
	3.1	x		4	4
	3.2		x	4	4.5
	3.3	x		4	3
	3.4		x	4	4
	3.5		x	3	3
	3.6	x		4	4
	3.7	x		3.5	3.5
	3.8		x	3	4
	3.9	x		4	3
	3.10		x	2	3.5
4	<b>Characterization and Taxonomy</b>				
	4.1		x	4	4
	4.2		x	4	3.5
	4.3		x	4.5	4
5	<b>Resistance and Breeding</b>				
	5.1		x	3	3
	5.2		x	1	1
	5.3		x	3.5	3.5
	5.4		x	2.5	4
	5.5		x	3.5	4
6	<b>Culturing HLB</b>				
	6.1		x	1.5	5
7	<b>Pathogens/Vector/Host Interactions</b>				
	7.1		x	4	3.5
	7.2		x	4	4
8	<b>Chemical Control</b>				
	8.1		x	4	4
	8.2		x	4	4
	8.3		x	2	4
	8.4		x	1.5	4.5
	8.5	x		4	3
	8.6		x	2	3
	8.7		x	3	3
	8.8		x	3	3
	8.9		x	3	3.5
9	<b>Biological Control</b>				
	9.1		x	3	3
	9.2		x	3	3
	9.3		x	2.5	3
	9.4		x	2	4
	9.5		x	3	3.5
	9.6		x	3	3.5
10	<b>Cultural Control</b>				
	10.1		x	5	4.5
	10.2		x	4	4
	10.3	x		3.5	3
	10.4		x	4	4
	10.5		x	2	2.5
	10.6		x	3	3
11	<b>Epidemiology</b>				
	11.1		x	2.5	2.5
	11.2		x	3.5	4
	11.3		x	3.5	4
	11.4		x	3	4
	11.5		x	3	3
	11.6	x		4.5	4
	11.7		x	3	3
	11.8	x		4	4
	11.9		x	2	4
12	<b>Transgenics</b>				
	12.1		x	3	4.5
	12.2		x	3	4.5
	12.3		x	3	4.5
	12.4		x	1.5	3
	12.5		x	3	4
13	<b>Genomics</b>				
	13.1		x	3.5	3.5
	13.2	x		5	5
	13.3		x	4	5
	13.4	x		4.5	3.5
	13.5		x	3.5	4
14	<b>Vector Biology</b>				
	14.1		x	3.5	4
	14.2		x	3	4
	14.3		x	3.5	4
15	<b>Fruit Yield and Quality</b>				
	15.1	x		4	3
	15.2	x		3.5	3.5
	15.3		x	3.5	3.5

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Research Recommendations: **Citrus Canker 2005**

- Remote Sensing
- Spectral Analysis
- Economics
- Survival of Xac
- Detection of Disease
- Differentiation
- Resistance
- Citrus Breeding
- Citrus Genetics
- Citrus Resistance
- Chemical Control
- Biological Control
- Cultural Control
- Pathogenesis
- Epidemiology
- Transgenics
- Genomics

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- **1 Chemical**

- 1.1 Evaluation of ISR/SAR etc. related to ccA
- 1.2 Application Methods for Chemicals – Aircraft et al.
- 1.3 Investigation of Curative and Preventative Properties of Microbicides
- 1.4 Combinations of Chemical Controls with Copper – IPM
- 1.5 Asian Citrus Leaf Miner
  - Evaluation of New and Existing Compounds: Vydate, E2Y, Copper GX
  - Pheromones monitoring and mating disruption
- 1.6 Resistance of Xac to copper
- 1.7 Testing of sanitizing compounds to pre- post-harvest, packing house
- 1.8 Search for systemic bactericide

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- **2 Cultural Control**

- 2.1 Effect of Irrigation and Spray Practices on Disease Increase
- 2.2 Optimization of Windbreaks
- 2.3 Pruning (disease control, dwarfing)
- 2.4 Field Susceptibility of Cultivars - Flush management
- 2.5 Nutrition
- 2.6 Defoliation
- 2.7 Orchard Management Systems
- 2.8 Mechanical Harvesting Impact
- 2.9 Protected Production (relocation, greenhouses: nursery vs grove)
- 2.10 Alternative Land Uses during Fallow Period
- 2.11 Investigation of Practices – foreign sources

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- **3 Biological Control**
  - 3.1 Xanthomonas Control with Bacteriophages to Decrease Inoculum
  - 3.2 Use of Antagonistic or Site-competitive Microorganisms
  - 3.3 Interaction of A and B Strains of Citrus Canker and Their Competition
  - 3.4 Microbial Community Phyloplane and Endophytes
  - 3.5 Citrus Leaf Miner
  - 3.6 Cross-protection using Avirulent Canker Strains
  - 3.7 Antagonistic Effects of HLB Controls

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- **4 Remote Sensing and Information/Tracking (GIS+) Systems**
  - 4.1 Proof of Concept of Spectral Analysis to Citrus Canker
  - 4.2 Application and Deployment
    - Low Level spectral Characteristics
    - Application to Finding Citrus Canker / Citrus Trees
    - Focus on Aircraft-based Hyperspectral Analysis

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- **5 Detection Technology**

- 5.1 Prove Canines can Differentiate Citrus Canker
- 5.2 Visual Detection – Sensitivity & Reliability
- 5.3 Electronic Noses - Pathways for Citrus Entry
- 5.4 Electronic Noses - Application to Citrus and Citrus Canker Detection
- 5.5 Quantitative PCR to Detect Non-culturable Citrus Canker
- 5.6 Detect Host Response prior to Lesion Development – microarrays et al.
- 5.7 High Throughput of PCR for Citrus Canker Detection
- 5.8 Improved Detection Sampling Designs
- 5.9 Nanotechnology
- 5.10 Field Deployable Rapid Detection Technology
- 5.11 Nursery Detection Technology

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#### 6 Citrus Resistance and Breeding

- 6.1 Knowledge of Pathogen-based Resistance
- 6.2 Genomic Comparisons - Resistance Responses
- 6.3 Citrus Resistance Triggers and Map-based Cloning
- 6.4 Generation of Resistant Germplasm
- 6.5 Performance of Resistant Cultivars from Worldwide Sources
- 6.6 Genetic Characterizations of Resistance in Citrus
- 6.7 Rutaceae Susceptibility to Citrus Canker
- 6.8 High Throughput/Improved Screening Systems
- 6.9 Determination Of Biological Elicitors of Plant Defenses
- 6.10 Lytic Peptides and Delivery Systems
- 6.11 Develop Markers For Selection In Breeding Programs Linked to Resistance
- 6.12 Differentially Expressed Genes - cca EST or cDNA Library
- 6.13 Exploitation Of Resistance Gene Candidate Sequences Already Cloned from Citrus
- 6.14 Classical Breeding Techniques for Resistance
- 6.15 Dwarfing Rootstocks
- 6.16 Foreign Exploration for Other Sources
- 6.17 Expedited Field Trials for Performance

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- **7 Differentiation / Characterization of Xac Strains**
  - 7.1 Standardization and Quality Assurance:  
Ring test and methods of certification; global web site
  - 7.2 Establish International Collections / Repositories  
Permanent Florida, national (Beltsville), and international location  
Funding and collection size
  - 7.3 Improve Rapid Strain Differentiation Techniques
  - 7.4 Strain Characterization for Origin  
Differentiation for host/pathogen interactions  
Creating marked strains

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- **8 Pathogenesis**
  - 8.1 Nature of Mesophyll Resistance to ccA
  - 8.2 Xanthomonas Genomics and Functional Analysis
    - Identification of genes necessary for
    - 1) infection and
    - 2) induction of resistance expression due to infection

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- **9 Survival**
  - 9.1 Survival of Bacterium in Packing Container
  - 9.2 Probability of Transmission from Fruit and or Plant Materials Disinfested
  - 9.3 Survival of Bacterium on Lesioned or Lesionless Plant Tissues
  - 9.4 Use Dilution Strength, Biodegradable, Bacteriocide
    - Develop all-purpose disinfectant

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- **10 Economics**

- 10.1 Economic Analysis

- Economic losses due to restricted movement

- Conclusive science to achieve a defensible position related to risk of fruit movement (risk assessment: Florida's white paper, in part)

- Economic analysis of lost markets (domestic and international)

- Economic benefit of defoliation vs tree removal

- Economic analysis of control measures

- Phytosanitary systems for fruit movement from quarantine areas

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- **11 Transgenics**
  - 11.1 Differentially Expressed Libraries to Identify Promoters
  - 11.2 Transgenic Citrus with Resistance Genes from Citrus and other Plants and Organisms
  - 11.3 Transformation System Development
  - 11.4 High throughput Screening
  - 11.5 Transgenic Rootstocks
  - 11.6 Use of Viral Vectors
  - 11.7 Interaction between Scion and Rootstock
  - 11.8 Site-directed Mutagenesis
  - 11.9 Novel Technologies

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- **12 Genomics**
  - 12.1 Differentially Expressed Libraries in Response to Asian Citrus Leaf Miner feeding
  - 12.2 Sequencing citrus genome

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- **13 Epidemiology**

- 13.1 International Field-scale Study (multinational):
  - Large vs small scale
  - Sampling Methods / Technology
  - Visual survey efficiency
  - Deployment of survey and sampling technologies (coordination)
  - Chemical of leafminer and Xac, windbreaks, weather forecast systems, defoliation, irrigation etc.
- 13.2 Meteorological Events and their Distance of Spread
  - Effects on development of disease
  - Evaluation in Different Cultural Settings
  - Local, international, greenhouse / laboratory
- 13.3 Latency Duration of Fallow
- 13.4 Isolation Distances for Nurseries
- 13.5 Alternative Distances and Timing

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13. 6 Control studies within an Endemic / Epidemic

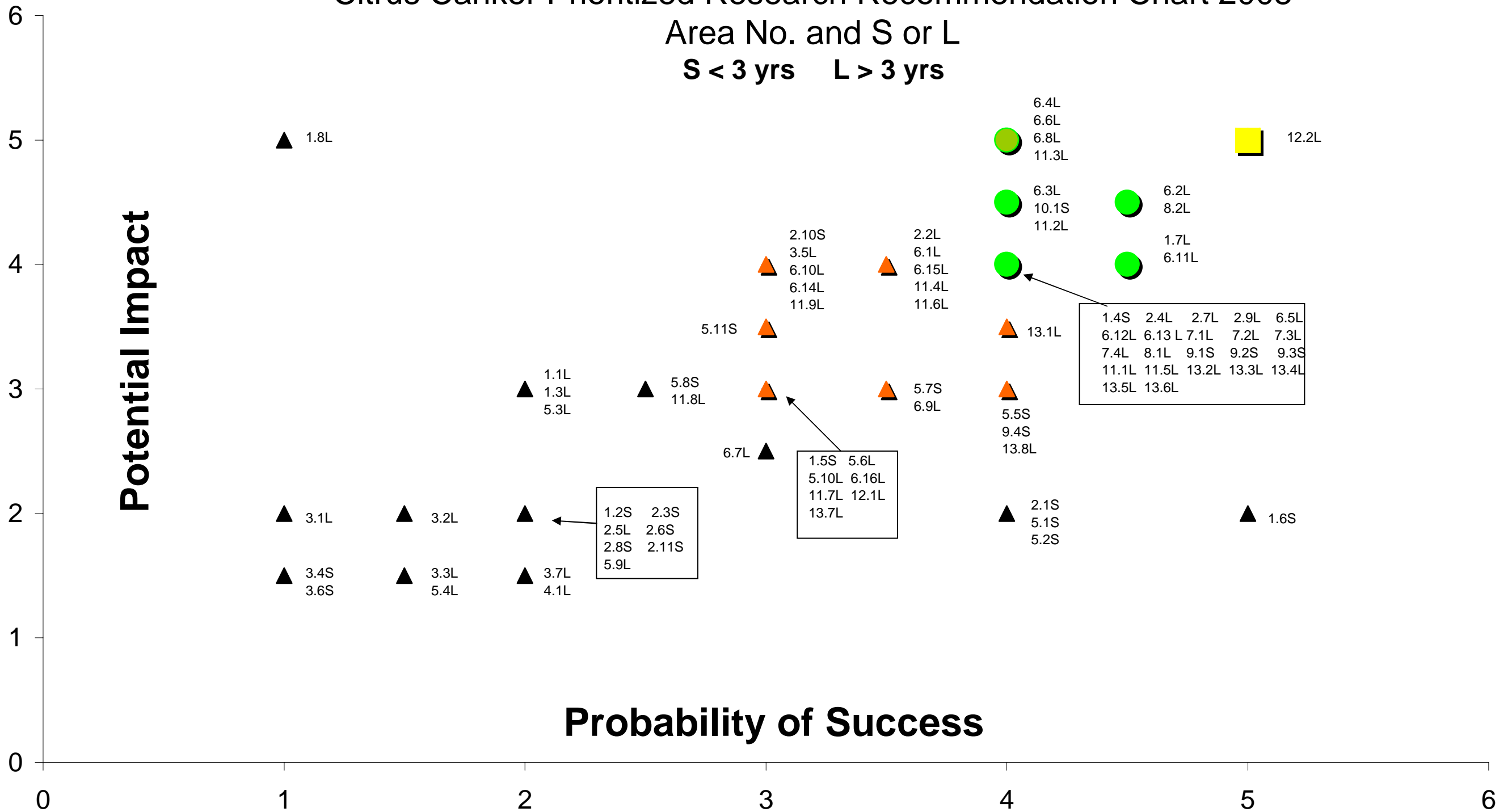
- Eradication Campaign – Epidemic
- Management – Endemic: surrogate organisms, environmental variations
- Application and impact of windbreaks, defoliation techniques
- Pre-eradication inoculum suppression techniques – defoliation, tarping
- 13.7 Insecticide / Microbicide / Surfactant Influences
  - Enhance disease expression on trap plants using surfactants
  - Cuticle studies for adjuvants and penetrants for systemic chemical delivery
  - Microbicide as prevention of inoculum transfer using local or systemic compounds
- 13.7 Damage Evaluation System

# Citrus Canker Prioritized Research Recommendation Chart 2005

Area No. and S or L

S < 3 yrs L > 3 yrs

Potential Impact



1.4S 2.4L 2.7L 2.9L 6.5L  
 6.12L 6.13L 7.1L 7.2L 7.3L  
 7.4L 8.1L 9.1S 9.2S 9.3S  
 11.1L 11.5L 13.2L 13.3L 13.4L  
 13.5L 13.6L

1.5S 5.6L  
 5.10L 6.16L  
 11.7L 12.1L  
 13.7L

1.2S 2.3S  
 2.5L 2.6S  
 2.8S 2.11S  
 5.9L

### Citrus Canker Prioritized Research Recommendation List

Scale is 1 (low) to 5 (high)

Area No.	Project	Short Term < 3yrs	Long Term > 3 yrs	Probability of Success	Potential Impact
1	<b>Chemical</b>				
	1.1		X	2	3
	1.2	X		2	2
	1.3		X	2	3
	1.4	X		4	4
	1.5	X		3	3
	1.6	X		5	2
	1.7	X		4.5	4
	1.8		X	1	5
2	<b>Cultural Control</b>				
	2.1	X		4	2
	2.2		X	3.5	4
	2.3	X		2	2
	2.4		X	4	4
	2.5		X	2	2
	2.6	X		2	2
	2.7		X	4	4
	2.8	X		2	2
	2.9		X	4	4
	2.10		X	3	4
	2.11	X		2	2
3	<b>Biological Control</b>				
	3.1		X	1	2
	3.2		X	1.5	2
	3.3		X	1.5	1.5
	3.4		X	1	1.5
	3.5		X	3	4
	3.6		X	1	1.5
	3.7		X	2	1.5
4	<b>Remote Sensing</b>				
	4.1		X	2	1.5
	4.2		X	1.5	2
5	<b>Detection</b>				
	5.1	X		4	2
	5.2	X		4	2
	5.3		X	2	3
	5.4		X	1.5	1.5
	5.5	X		4	3
	5.6		X	3	3
	5.7	X		3.5	3
	5.8	X		2.5	3
	5.9		X	2	2
	5.10		X	3	3
	5.11	X		3	3.5
6	<b>Resistance</b>				
	6.1		X	3.5	4
	6.2		X	4.5	4.5
	6.3		X	4	4.5
	6.4		X	4	5
	6.5		X	4	4
	6.6		X	4	5
	6.7		X	3	2.5
	6.8		X	4	5
	6.9		X	3.5	3
	6.10		X	3	4
	6.11		X	4.5	4
	6.12		X	4	4
	6.13		X	4	4
	6.14		X	3	4
	6.15		X	3.5	4
	6.16		X	3	3
	6.17		X	4	4
7	<b>Differentiation</b>				
	7.1		X	4	4
	7.2		X	4	4
	7.3		X	4	4
	7.4		X	4	4
8	<b>Pathogenesis</b>				
	8.1		X	4	4
	8.2		X	4.5	4.5
9	<b>Survival</b>				
	9.1	X		4	4
	9.2	X		4	4
	9.3	X		4	4
	9.4	X		4	3
10	<b>Economics</b>				
	10.1	X		4	4.5
11	<b>Transgenics</b>				
	11.1		X	4	4
	11.2		X	4	4.5
	11.3		X	4	5
	11.4		X	3.5	4
	11.5		X	4	4
	11.6		X	3.5	4
	11.7		X	3	3
	11.8		X	2.5	3
	11.9		X	3	4
12	<b>Genomics</b>				
	12.1		X	3	3
	12.2		X	5	5
13	<b>Epidemiology</b>				
	13.1		X	4	3.5
	13.2		X	4	4
	13.3		X	4	4
	13.4		X	4	4
	13.5		X	4	4
	13.6		X	4	4
	13.7		X	3	3
	13.8		X	4	3