Proceedings of the International Citrus Canker and Huanglongbing Research Workshop

November 7-11, 2005
Orlando, Florida

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Second International Citrus Canker and Huanglongbing Research Workshop
Orlando, Florida
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2005

Mission Statement: Disseminate information on research progress since the First International Canker Research Workshop, held June 2000, and renew priorities for research based on the current and future status of canker in the Florida citrus industry and the global citrus industry. Assemble international researchers with specific expertise on Huanglongbing to discuss the current status of knowledge and to extract information relative to the current Florida situation.

The workshop is an invitational meeting of leading scientists from the international research community, scientists and administrators from state and federal regulatory agencies, scientists involved in education and the eradication program, and representatives from the Florida citrus industry to exchange information on citrus canker and Huanglongbing.

Invited scientists and participants are charged with developing a prioritized list of research recommendations. This information will be used by the research, regulatory, and citrus industry communities to provide input into the current Citrus Canker Eradication Program, opportunities for eradication/suppression and control of Huanglongbing and to make recommendations for the most appropriate direction for continued research on citrus canker and new research efforts on huanglongbing.

Steering Committee Co-chairmen: Andy LaVigne, Tim Gottwald, Jim Graham, Phil Berger, Wayne Dixon

Agenda

MONDAY, 7 Nov 2005

7:00 – 9:00 PM Workshop Welcome Reception

TUESDAY NOVEMBER 8, 2005: CURRENT STATUS OF CITRUS CANKER

7:30 – 8:00 AM Registration

8:00 – 8:15 AM Welcome/Introduction Andy LaVigne, CEO Florida Citrus Mutual
Mission/Goals/Objectives Tim Gottwald
Housekeeping/Rules of the House Wayne Dixon

Morning Session: Wayne Dixon - Moderator

8:15 - 8:45 AM Intro to Citrus canker situation in Florida
C1 –Citrus Canker: "Doing Battle with the Beast for Nearly a Century" – **T. Gottwald**

8:45 – 9:00 AM Current Status of Citrus Canker Control Program in São Paulo, Brazil
C2 – Citrus Canker Eradication Program in Sao Paulo State, Brazil - C. A. Massari, **J. Ayres**, J. Belasque Jr., R. B. Bassanezi, J. C. Barbosa

9:00 – 9:45 AM Epidemiology:
C3 - Estimating the Increase and Spread of Citrus Canker Caused by the Interaction of Pedestrian Versus Catastrophic Weather Events, Humans, and Bad Luck – **T. Gottwald**, J. Graham, T. Riley, X. Sun, G. Hughes, F. Ferrandino, E. Taylor, C. Bock, M. Irey, C. Gilligan, and B. Seem
C4 –Wind Speed Effects on the Dispersal of *Xanthomonas axonopodis* pv *citri* and Infection of Grapefruit Leaves – **C. Bock**, P. E. Parker, A. Z. Cook, T. R. Gottwald
C5 –Post-hurricane Analysis of Citrus Canker Spread and Progress Towards the Development of a Predictive Model for Future Weather Related Spread – **M. Irey**, T. Gottwald, J. Graham, G. Carlton, T. Riley

9:45–10:15 AM Epidemiology of Citrus Canker in Brazil with and without Asian Citrus Leaf Miner
C6 – Effects of Injuries Caused by Different Instars of Phyllocnistis citrella on Infection by Xanthomonas axonopodis pv citri - **J. Belasque**, W. C. Jesus Jr., R. S. C. Christiano, L. Amorim, A. Bergamin-Filho, J. R. P. Parra
C7 - Spatial Pattern Analysis of Citrus Canker Infected Plantings in Sao Paulo Brazil and Implication of the Asian Leafminer on Potential Dispersal Processes - **T. R. Gottwald,** R. B. Bassanezi , L. Amorim and A. Bergamin-Filho
C-8 – Distance of Spread of Asian Citrus Canker From Source of Infection in Commercial Plantings in Sao Paulo, Brazil – L. Amorim, W. C. Jesus Jr., **R. B. Bassanezi**, A. Bergamin-Filho, T. R. Gottwald

10:15-10:30 AM Break - Refreshments provided by the Florida Citrus Industry

Mid-Morning Session: Tim Schubert - Moderator

10:45 – 11:15 AM Survival of Canker Bacterium in the Environment
C9 – Use of Green Fluorescent Protein (Gfp) to Study Survival of *Xanthomonas axonopodis* on Citrus Plant Surfaces - J. Cubero, **J. Graham**, T. Gottwald, A. Redondo, M. Dekkers, Y. Zhang, J. B. Jones, M. Wilson
C10 – Rational, Techniques and Efficacy of Sanitation in Canker Eradication – **T. Schubert**
C11 –Standardization of Diagnosis for Citrus Bacterial Canker: the EPPO Protocol and Its Use for Detection in Citrus Fruits – **M. Lopez**, J. Cubero, P. Llop, J. Penalver, J. M. Quesada, C. Morente, V. Catara

11:15 AM – 12:15 PM Integrated Control Studies in Brazil and Argentina and Control Studies in Florida with CBS
C-12 –Epidemiology, Management and Effect on Yield of Citrus Canker Under Endemic Conditions in Parana, Brazil – **A. Bergamin-Filho**, F. Behlau, J. Belasque, Jr., L. Amorim, R. P. Leite, Jr., T. R. Gottwald
C13 – Integrated management of citrus canker in southern Brazil - **Rui Leite**

C14 - Citrus Canker Sanitation in Groves by Drastic Pruning – **J. Belasque**, J., Ayres, A.J., Gimenes-Fernandes, N.
C15 –Chemical Control of Citrus Canker in Lemons (Citrus limon, (L.)Burm. F) in Tucuman, Argentina – **B. Stein**, J. Ramallo, L. Foguet, M. Morandini
C16 – Control of Asiatic Citrus Canker and Citrus Bacterial Spot with Bacteriophages in Florida – **B. Balogh,** J. B. Jones, R. E. Stall, J. R. Dilley, H. D. Yonce, B. I. Canteros, A. M. Gochez

12:15 – 1:30 PM Lunch provided by the Florida Citrus Industry

1:30 – 2:00 PM Economic analysis of citrus canker eradication
C17 – Economic Analysis of Florida's Citrus Canker Eradication Program – **T. H. Spreen**, M. L. Zansler, R. P. Muraro
C18 – Implementation of the Citrus Canker Eradication Program: Lessons Learned from the Stop-and-Go Approach to Eradicating Exposed Trees – **M. L. Zansler,** T. H. Spreen, R. P. Muraro

2:00 – 3:15 PM Methods for Detection, Enumeration and Characterization of *Xanthomonas axonopodis* pv**. *citri***
C19 – Real Time PCR and Allelic Discrimination to Detect, Enumerate and Differentiate *Xanthomons axonopodis* Strains Causing Citrus Canker - J. Cubero, **J. Graham**
C 20 - A 20-Minute Real-time TaqMan® Assay for Fast and Reliable Diagnostics of Citrus Canker - R. Dorsey, M. Karavis, S. Shah, M. Goode, V. Mavrodieva, **L. Levy**
C21 – Rapid Genetic Typing of Strains of Xanthomonas citri, the Causal Agent of Citrus Canker in Florida – **Y-P. Duan**, D. Gabriel, B. D. Sutton, X. Sun, T. Schubert, W. Dixon
C22 – Detection of Manatee Genotypes of Citrus Canker Bacteria in Hillsborough Co., Florida – **X. Sun**, D. Jones, Y-P. Duan
C23 – Using PCR for Detection and Quantification of Xanthomonas axonopodis pv citri in wind driven splash – **C. Bock**, P. E. Parker, T. R. Gottwald, V. A. Mavrodieva, L. Levy

3:15-3:30 PM Break refreshments provided by the Florida Citrus Industry

Late Afternoon Session: Pat Barkley - moderator

3:30 - 4:30 PM Survey Methods and Integration of Diagnostic Methodologies: Strengths and Weaknesses
C24 - Survey Strategies Utilized in Commercial Groves and Residential Properties for Citrus Canker Detection - **T. Riley**, G. Carlton
C25 – Florida Grower Citrus Canker Self-Inspection Program – **H. Chamberlain**, L. W. Timmer, J. H. Graham
C26 – Old versus new canker eradication strategy in São Paulo, Brazil. **J. Belasque Jr.**, A. Bergamin-Filho, L. Amorim, R.B. Bassanezi

4:30 – 5:15 PM Xanthomonas Genetics and Phylogeny
C27 – An Overview of the *Xanthomonas axonopodis* pv *citri* Genome – **J. Hartung**
C28 – Identification of an Avirulence Gene in *Xanthomonas axonopodis* pv. *citri* Strain A – M. Rybak, G. V. Minsavage, R. Stall, **J. Jones**
C29 – Genetic Diversity and Worldwide Proliferation of Citrus Bacterial Canker Pathogens Identified in Historic Specimens – **J. Hartung**, W. Li, Q. Song, R. Brlansky

5:15 PM Adjourn – Steering Committee Meeting - Andy LaVigne, Tim Gottwald, Jim Graham, Phil Berger, Wayne Dixon

6:00-7:00 PM Poster Session on Citrus Canker and HLB– Beer and Bull

7:00 PM Dinner - Provided

WEDNESDAY NOVEMBER 9, 2005: CONCLUSION OF CITRUS CANKER STATUS AND CURRENT STATUS OF HUANGLONGBING-

Morning Session: Bill Dawson - moderator

8:00-8:15 AM Announcements – Gottwald/Dixon

8:15 – 10:15 AM Genetics of Host and Pathogen Interactions and Determinants of Resistance (continued)

C30 - Genetic Studies of Resistance to Citrus Canker – **F. G. Gmitter Jr.**, A. Ahmed, Y. Choi, J. Jones, G. Moore, C. Chen, and H. Shu

C31 – Moving citrus canker resistance from kumquat into triploid acid-fruit and mandarin hybrids via interploid crosses – Z. Viloria, J. Grosser, **J. Graham**

C32 – Genetics of Citrus Canker Host/Pathogen Interactions – **D. Gabriel**, Y-P. Duan, B. El-Yacoubi, A. El-Saadi

C33 – Rapid Screening of Anti-bacterial Gene Products in Citrus Trees Using a CTV-Based Transient Expression Vector – **W. Dawson**, A. S. Folimonov, S. Y. Folimonova, M. Dekkers, A. Redondo, J. Graham

C34 – Production of Transgenic Plants Expressing the NPR1 Defense Gene to Develop Resistance to Citrus Canker – **V. J. Febres**, G. Moore

C35 – Enhanced Resistance to Citrus Canker in Transgenic Sweet Orange Plants Expressing the Antibacterial Peptide Sarcotoxin IA – **J. Bespalhok**, L. Gonzaga, A. Kobayashi, I. Mitsuhara, H. Molinari, R. Leite, L. Meneguim, Y. Ohashi, S. Natori, L. Vieira

10:15 – 10:30 AM Break refreshments provided by the Florida Citrus Industry

10:30 – 10:45 AM Grove and Packinghouse Sanitation

C36 – Protocols for Orchard and Packinghouse Sanitation to Qualify for Fresh Fruit Shipments – **T. Schubert** and C. Davis

Mid-Morning Session: John da Graca - Moderator

10:45 – 11:15 AM HLB: Biology and Overview The World Situation

H1 - Huanglongbing: Biology and Overview **– John da Graca**

11:15 – 12:00 PM HLB in Sao Paul: Challenges and achievements

H2 – Huanglongbing in Sao Paulo State, Brazil: Challenges and Achievements, March 2004-September 2005 **- Josy Bové**, Antonio J. Ayres

12:00 – 1:15 PM Lunch provided by the Florida Citrus Industry

Afternoon Session: Steve Garnsey - Moderator

1:15 – 1:30 AM Introduction to HLB situation in Florida

H3 – The Discovery of Huanglongbing in Florida - **S. Halbert**

1:30 – 2:00 PM Current Status of HLB in São Paulo, Brazil: Eradication Efforts/plan

H4 - Current Status of HLB in São Paulo, State Brazil: Eradication Efforts/plan **J. Ayres**

H5 - Huanglongbing: Its Possible Origins, Collaborative Research in Southeast Asia, and Developing Incursion Management Plans for Australia – G. A. C. Beattie, D. J. Mabberley, P. Holford, **P. Broadbent**, P. DeBarro

1:45 - 2:15 PM HLB Epidemiology and Survey in Multiple Locations

H6 – Huanglongbing epidemiology: Tracking the Dragon Through Time and Space – **T.R. Gottwald**

H7 – Spatial Distribution of Huanglongbing Symptomatic Trees in Citrus Groves in Sao Paulo, Brazil - **R. Bassanezi**, L. A. Busato, A. Bergamin-Filho, L. Amorim, T. R. Gottwald

2:15 – 3:15 PM Detection and Diagnostics

H8 - Diagnostics of huanglongbing: detection of the causal liberibacters, *Candidatus Liberibacter asiaticus*, *Ca*. *L. africanus*, and *Ca*. *L. americanus,* in plants and insects by electron microscopy, DNA hybridization, and PCR. **– J. M. Bové**

H9 – Comparison of Methods for the Detection of Candidatus *Liberibacter Asiaticus* in Plant Samples – **J. S. Hartung**, W. Li, L. E. Levy

H10 – Development of Multiplex Real-Time PCR for Detection and Identification of Candidatus *Liberibacter* Species Associated with Citrus Huanglongbing – **W. Li**, D. C. Teixeira, J. S. Hartung, L. Levy

H11 – Detection and Identification of Citrus Huanglongbing (Greening) in Florida, USA – **B. D. Sutton**, Y-P. Duan, S. Halbert, X. Sun, T. Schubert, W. Dixon

3:15 3:30 PM Break refreshments provided by the Florida Citrus Industry

3:30-3:45 PM Diaphorina: Biology, life cycle, sampling and implications

H12 – Overview of the Asian Citrus Psyllid – **D. Hall**

3:45 -4:15 PM HLB Current Knowledge on Control Strategies

H13 – Studies on *Campylomma chinensis* Schuh, a Potential Biocontrol Predator of *Diaphorina citri* Kuwayama – **Z. Wu**

H14 – C-22 Management of Asian citrus psyllid on citrus with ADMIRE PRO, PROVADO, and TEMIK **J.Bell,** L.Hall, R.Morris, and M.Toapanta

4:15 – 4:45 PM Genetics, Resistance/Tolerance of Host and Pathogen Interactions

H15 – Strategies for Sequencing the Genome of Liberibacter, an Uncultured Select Agent – **D. Gabriel**, Y.-P. Duan, J. Reddy

H16 – Screening of anti-bacterial peptides in citrus trees for activity against Candidatus *Liberibacter asiaticus* – **J.S. Hartung**, A.S. Folimonov, S.Y. Folimoinov, and W.O. Dawson

4:45 - 5:15 PM Genome Sequencing of Liberibacter – Need for an international project?
Moderator Dean Gabriel
Panel Members – Josy Bove, John Hartung, Yong-Ping Duan, Bill Dawson

5:15 PM Adjourn – Steering Committee Meeting - Andy LaVigne, Tim Gottwald, Jim Graham, Phil Berger, Wayne Dixon

7:00 PM Dinner – On your own. Suggestions provided

THURSDAY NOVEMBER 10, 2005: NEW PRIORITIES, NEW APPROACHES FOR BOTH CITRUS CANKER AND HLB

Panel Discussion Sessions

8:00 – 9:45 AM Citrus Transgenics, and GMOs for both Canker and HLB - Discussion Panel
John Hartung Moderator – 10 min overview
Panel Members – Bespalhok, Fred Gmitter, Dean Gabriel, John Hartung

9:45 – 10:00 AM Break refreshments provided by the Florida Citrus Industry

10:00 – 11:00 AM Canker Epidemiology in Eradication vs. Endemic Scenarios – Discussion Panel:
Armando Bergamin Moderator – 10 min overview
Panel Members - Tim Gottwald, Pat Barkley, Rui Leite

11:00 –12:00 PM Canker Integrated Control Measures:
Exclusion and Sanitation in Nurseries and Groves – Discussion Panel:
Pete Timmer Moderator - 10 min overview
Panel members: Rui Leite, Jim Graham**,** Juliano Ayers, Tim Schubert

12:00 – 1:15 PM Lunch provided by the Florida Citrus Industry

1:15 – 2:15 PM Canker: Adoption of Cultural Control Methods — Discussion Panel:
Jim Graham Moderator
Tim Gottwald, Pete Timmer, Rui Leite, Jonathan Crane, Tim Schubert

2:15 – 3:00 PM New Chemical Controls on the Horizon – Discussion Panel:
Pete Timmer Moderator
Jim Graham, Bond McGinnes (Dupont)

3:00 – 3:15 PM Break refreshments provided by the Florida Citrus Industry

3:15 – 4:15 PM HLB Integrated Control Measures:
Exclusion, Nursery certification, vector control and eradication– Discussion Panel:
David Hall Moderator - 10 min overview
Panel members: Mike Irey, John daGraca, Tim Gottwald, Josy Bové, Juliano Ayers, Steve Garnsey, Michael Rogers

4:15 – 4:45 PM Capturing the salient points of current citrus canker knowledge – Tim Schubert

4:45 – 5:15 PM Capturing the salient points of current HLB knowledge – John daGraca

5:15 PM Adjourn – **Steering Committee Meeting -** Andy LaVigne, Tim Gottwald, Jim Graham, Phil Berger, Wayne Dixon

6:00 - 7:00 PM Poster Session – Beer and Bull

7:00 PM Dinner - Provided

FRIDAY NOVEMBER 11, 2005: DISCUSSION OF RESEARCH PRIORITIES

8:00-8:15 AM Announcements – Gottwald/Dixon

8:15 – 10:15 AM Update of Research Priorities:
M**oderated by:** Tim Gottwald, Wayne Dixon, Phil Berger
Wayne Dixon: Review of Research Priorities from 1st International Citrus Canker Research Workshop

10:15 – 10:30 AM Break refreshments provided by the Florida Citrus Industry

10:30 – 12:00 AM HLB: Establishment of Research Priorities
M**oderated by:** Tim Gottwald, Wayne Dixon, Phil Berger

12:00 – 1:15 PM Lunch provided by the Florida Citrus Industry
Steering Committee meets to prepare for afternoon
Written question forms made available to all for afternoon session

1:15 –2:45 PM Further Discussion and Finalization of Research Priorities

2:45 – 3:15 AM Sources of Funding for Research Priorities
Moderated by: Andy Lavigne, Harold Browning, Rick Bennett, Rick Dunkle.

3:15 – 3:30 PM Break refreshments provided by the Florida Citrus Industry

3:30 – 4:30 PM Industry and Media Overview and Question/Answer Session
Discussion Panel - Moderator: Andy LaVigne
Presentation of Research Priorities List – Wayne Dixon
Dixon and LaVigne to explain rules for questions.
Panel Members: Tim Schubert, Tim Gottwald, Jim Graham, Dean Gabriel, David Kaplan, John Hartung (Phil Berger), Josy Bové, John daGraca
Panel members to respond to written questions for the audience

4:30 PM Workshop Adjourns

Poster Session Contributions:

P1 – Citrus Canker exposure expansion in residential areas of Palm Beach County after Year 2004 hurricane season – **L. J. Crespo**, G. J. Monaghan, R. Miranda, M. J. Fagan

P2 – Success of program control results based on increased public education efforts (waiver-runners) – **L. J. Crespo**, G. J. Monaghan, R. Miranda, M. J. Fagan

P3 – Citrus canker exposure expansion as consequence of three years of court constraints – **L. J. Crespo**, G. J. Monaghan, R. Miranda, M. J. Fagan

P4 - Functional Genomics and Diagnostic of Xanthomonas axonopodis pv. citri Towards Pathogenicity-Related Genes. A. M. do Amaral, J. C. Baptista, C. B. Souza, F. V. Winck, J. Roncoletta, **H. D. Coletta Filho**, M. A. Takita, M. A. Machado.

P5 – The distribution of citrus canker in Emerald, Australia and bacterial survival in citrus trash – **C. F. Gambley**, M. Benham, K. Parmenter, A. K. Miles, V. J. Doogan, M. Ramsden, P. J. L. Whittle

P6 – Evaluating potential alternative hosts of citrus canker – **D. L. Hailstones**, M. P. Weinert, M. W. Smith, A. Ghalayini, C. F. Gambley

P7 – The truth and facts pertaining to the handling of the citrus canker outbreak in Emerald, Australia from an Emerald citrus grower's perspective – **M. Matthews**, C. Pressler, J. Pressler

P8 – A concise history of citrus and citrus canker in Texas – **M. Skaria**, J. V. da Graca, J. V. French, Paul E. Parker, R. A. Vlasik

P9 – Microarray expression profiling of Nagami kumquat during its incompatible interaction in response to canker – **A. Ahmed**, J. B. Jones, G. Moore, F. G. Gmitter, Jr.

P10 – Quantitative trait linkage (QTL) mapping for resistance to citrus canker within citrus – **Y. A. Choi**, C. Chen, S. Huang, F. G. Gmitter

P11 – A comparison of image analysis and visual assessment of citrus symptoms – **C. H. Bock**, P. E. Parker, T. R. Gottwald

P12 – Construction and deployment of mobile containment greenhouse/laboratory – **P. E. Parker**, T. R. Gottwald, L. Levy, A. Z. Cook

P13 – The change in quantity of bacteria of *Xanthomonas axonopodis* pv *citri* dispersed down wind from canker-infested grapefruit trees during a wind/rain event – **C. H. Bock**, P. E. Parker, T. R. Gottwald

P14 - Effective detection and genome sequencing approaches of *Candidatus Liberibacter* sp. causing Huanglongbing (HLB) in São Paulo, Brazil. **H.D. Coletta-Filho**, E.F. Carlos, M.A. Takita, M.L.P.N. Targon, K.C. S. Alves, A.M. do Amaral, M.A. Machado

P15 - Citrus Defoliation: A Strategy to Prevent the Spread of Citrus Canker in Florida – M. Toapanta, **H. L. Chamberlain**, D. Schobert

P16 - Protoplast Transformation and Regeneration of Transgenic 'Hamlin' Sweet Orange Plants Containing a cDNA Xa21 *Xanthomonas* Resistance Gene and GFP – **A. A. Omar**, J. Graham, J. Grosser

P17 - Current Research on the Efficacy and Timing of Pesticide Applications for Suppression of Asian Citrus Psyllid (*Diaphorina citri*) Populations in Florida Citrus. **Michael E. Rogers**

P18 - Screening citrus germplasm for resistance to *Xanthomonas axonopodis* pv. *citri*– **G. McCollum**, K. Bowman, T. Gottwald

P19 - Transformation of citrus cultivars with antimicrobial genes for potential resistance to citrus canker (*Xanthomonas axonopodis* pv *citri*). **J.R. Gonzalez**, J. Graham, T.E. Mirkov

P20 – The *hms* Locus in *Xanthomonas spp*.: a Candidate for Biofilm Formation and Virulence. **V. Chow**, G. V. Minsavage, J. B. Jones, T. Romeo, J. F. Preston

P21 - Progress Towards the Development of an Effective Risk Analysis Process for the Florida Citrus Nursery Industry to Mitigate the Impact of Citrus Canker and Huanglongbing. - **M. S. Irey**, J. H. Graham, and T. R. Gottwald

P22 – Incidence of *Diaphorina citr*i (Hemiptera:Psyllidae) and its natural enemies in Puerto Rico and Florida. **R. Pluke, A. Urbaneja** and **P. Stansly**

P23 – Contribution of Predation and Parasitism to Mortality of Citrus Leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) Populations in Florida.

P24 – Reducing Canker Risk through Biological Control of Citrus Leafminer. **Phil Stansley**, **Run Nguyen**

The Second International Citrus Canker and Huanglongbing Research Workshop

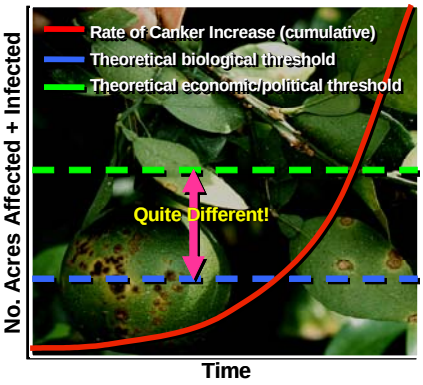
Contributed Papers on Citrus Canker C1-C36

C1 – Citrus Canker: "Doing Battle with the Beast for Nearly a Century"

Tim Gottwald, USDA, ARS, USHRL, Fort Pierce, Florida

Asiatic citrus canker (ACC) has a long history in Florida. The disease was first found around 1910 and spread throughout the southeastern US on imported citrus seedlings from Japan. After an extensive eradication program, canker was declared eradicated from Florida and the adjacent states in 1933. Citrus canker was discovered again in Manatee Co. Florida south of Tampa Bay in 1986, and was declared eradicated by 1994. Three years later the disease re-emerged on the west coast of Florida where the 1980s outbreak had occurred. Concurrently, a new epidemic of ACC was concurrently discovered in urban Miami in 1995, with an estimated introduction some time in 1992 or 1993. When first detected in Miami in 1995, the epidemic area was approximately 36.3 km^2 (14 mi^2) of largely residential properties southwest of the Miami International Airport. Over the past decade ACC has spread throughout most production areas of Florida. In response to the 1995 detection of citrus canker, a cooperative state/federal citrus canker eradication program (CCEP) was established between the Florida Department of Agriculture and Consumer Services (FDACS), Division of Plant Industry (DPI) and the USDA, Animal and Plant Health Inspection Service (APHIS).

The introduction of the Asian leafminer which exacerbates ACC outbreaks, combined with numerous hurricanes, tropical storms, severe local weather, mechanical-human movement of plant material, legal challenges curtailing eradication for multiple years, and inattentiveness by some to sanitation protocols, has lead to the critical situation currently facing the Florida citrus industry. As eradication has progressed, various unique isolates of the causal bacteria, *Xanthomonas axonopodis* pv *citri* (Xac) have been discovered and characterized. The presence of multiple isolates is indicative of multiple introductions of Xac and demonstrates the porosity of international borders even when subject to vigilant surveillance and regulatory controls to prevent importation of exotic pests and pathogens and further demonstrates the necessity for perpetual post-introduction surveillance systems, such as the residential sentinel survey and the multiple commercial survey methods presently deployed.



A challenge for any eradication program is to develop criteria that accurately evaluate the effectiveness of eradication. One critical criterion is the continual reevaluation of the incidence and distribution of the disease. For the ACC eradication campaign in Florida, manpower and resources became limiting in 2005 following the 2004 hurricane season which resulted in numerous outbreaks of canker in previously disease-free areas of the commercial citrus industry. New survey strategies were developed including, the targeted grove survey (meteorological event driven), sentinel grove survey, and the commercial producer self survey. These were combined with existing grove, residential sentinel, and delimiting surveys to rapidly and systematic blanket the commercial industry to find and delimit post-hurricane infections. Another and perhaps more important criterion to evaluate, is the threshold of disease beyond which eradication is no longer biologically possible. However, there are other thresholds that may be even more critical from a commercial industry perspective, such as economic, political and social thresholds for disease tolerance. The relative importance of the socio-economic/political thresholds and their relationship to one another and to the biological threshold can change depending upon the criteria used to evaluate them. These and other eradication issues will be discussed.

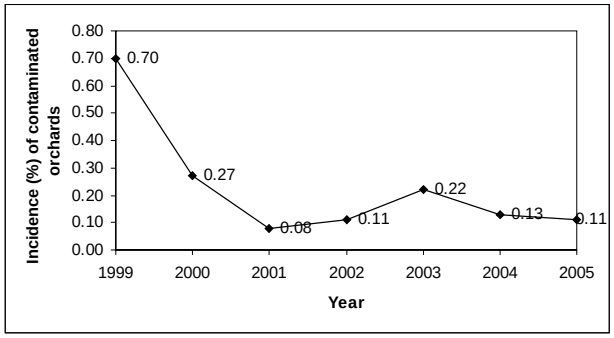
C-2 Citrus Canker Eradication Program in São Paulo State, Brazil

Massari, C.A.[1], **Ayres, A.J.**[1], Belasque Júnior, J.[1], Bassanezi, R.B.[1], Barbosa, J.C.[2]

[1]Fundecitrus, Araraquara, Brazil; [2]FCAV/UNESP, Jaboticabal, Brazil

Citrus canker was first reported in Brazil in 1957, in Presidente Prudente county, São Paulo State. Since then an eradication program was started and quarantine efforts have been applied. After the introduction of citrus leafminer (*Phyllocnistis citrella*, Stainton), in 1996, a higher number of citrus canker foci was observed and, by consequence, the eradication methodology was changed in 1999 by a State law. In São Paulo State the eradication program is made by efforts from the Federal and State Governments and the citrus growers, organized in the Found for Citrus Plant Protection (Fundecitrus). To find contaminated groves in the State, Fundecitrus makes an annual survey in all trees of 10% of the commercial blocks (with more than 199 citrus trees). Based on the distribution of contaminated groves in the State, the Eradication Program determines the inspection actions in the citrus production area. Depending on contamination incidence, all citrus blocks of some counties are inspected more than once a year. Inspections made by citrus growers are another form to find contaminated citrus trees in commercial blocks. Inspections by Fundecitrus are done also in citrus nurseries and urban areas. Contaminated citrus blocks are simultaneously observed by three teams of inspectors. When there are more than 0.5% of infected trees, all trees in the block, infected and non-infected, are eradicated. If there are less or equal than 0.5% of infected trees, the infected trees and the non-symptomatic trees, in 30 meters of radius, are eradicated. There are quarantine restrictions and new citrus plantations are prohibited in the eradicated area up to two years after eradication. New inspections are done periodically in the eradicated area during the quarantine time. From 1999 to 2004 a total of 3,832,309 citrus trees were eradicated in commercial blocks, 416,797 in citrus nurseries, and 438,363 in non-commercial blocks. In 2005 were eradicated, up to May, 138,397 commercial trees and 113,326 non-commercial trees. Any contaminated citrus nursery wasn't found in this year (January to May). The incidence of contaminated commercial citrus blocks, found during the surveys, is presented in the Figure 1. Weather conditions and number of inspectors (number of inspected trees) – that directly reflects the eradication efforts – are the most important factors that affect the citrus canker infection in the State.

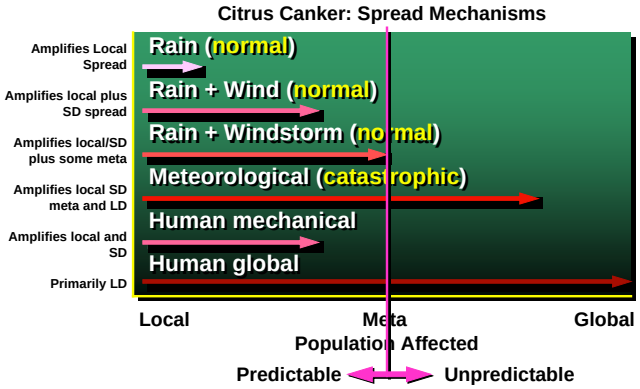
Figure 1. Incidence of citrus blocks infected with citrus canker in São Paulo State and South of Minas Gerais State determined by surveys in all the trees of 10% of the commercial citrus blocks.

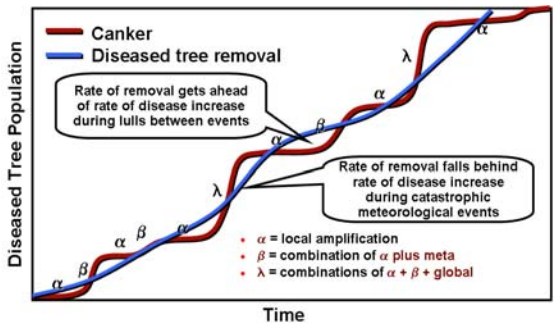


C-3 - Estimating the Increase and Spread of Citrus Canker Caused by the Interaction of Pedestrian Versus Catastrophic Weather Events, Humans, and Bad Luck.

Tim Gottwald, Jim Graham, Tim Riley, Xiaoan Sun, Gareth Hughes, Frank Ferrandino, Earl Taylor, Clive Bock, Michael Irey, Christopher Gilligan, and Bob Seem.

The bacteria, *Xanthomonas axonopodis* pv. *citri* (Xac), that causes Asiatic Citrus Canker (ACC) can move in any of a variety of modes in the presence of free moisture. From a meteorological point of view, gentle rain, rain with wind, rain storms, tropical storms, and hurricanes can all disperse Xac inoculum. The preceding series of meteorological events are increasingly more effective at dispersing inoculum over greater distances. If inoculum is maintained in a moist condition, mechanical spread and subsequent infection can occur also over a range from within tree to very long distance dispersal via human transport. Human assisted dispersal can also occur by movement of infected plant material, which can range from very short distance (local) to extremely long range (global = among countries and continents). From an epidemiological point of view, epidemics of ACC are composed of a series of discontinuous pulses of inoculum that first introduce Xac to the host population, then a combination of multiple meteorological and mechanical events further dispersing inoculum and exacerbating the epidemic. However, dispersal events vary greatly in distance and quantity of inoculum dispersed. As a result, the temporal dynamics of citrus canker within individual trees, within individual plantings, and regionally is essentially a stair-step function with the 'rise' of the step directly related to the intensity of the event and the 'run' directly related to the time between events. Another complicating factor is the incidence and severity of Asian leafminer. This insect has greatly exacerbated ACC epidemics in both Florida and Brazil by causing prodigious production of inoculum and increasing the number of infection courts that greatly enhance and prolong host susceptibility and when infected, result in further increases in inoculum production. The interaction between leafminer, Xac, and meteorological events results in greatly increased bacterial dispersal and subsequent bacterial infection gradients. Numerous storm and mechanical events have contributed to the spatial distribution and patterns of spread of ACC in Florida. The three hurricanes and one tropical storm that crossed the Florida peninsula during 2004 exacerbated preexisting ACC infections and established numerous new infections. The various affects of rain intensity, weather frontal boundaries, tornados, and other meteorological events will be discussed.





C-4 Wind speed effects on the dispersal of *Xanthomonas axonopodis* pv *citri* and infection of grapefruit leaves

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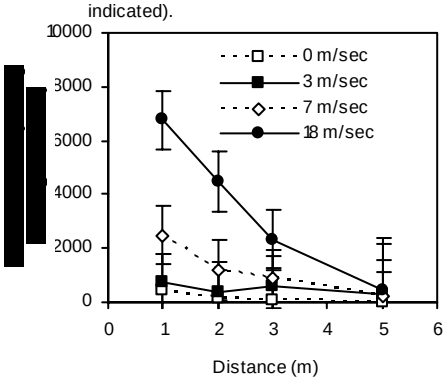
Citrus canker (caused by *Xanthomonas axonopodis* pv. citri, *Xac*) is an important disease limiting yield and marketability of citrus in several tropical and sub-tropical regions. In several afflicted areas, including in Florida (1), canker eradication has been instigated. The objective of this study was to investigate how wind affects the processes of *Xac* dispersal and citrus infection.

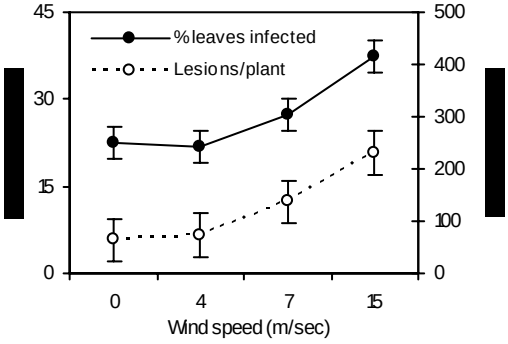
Wind speeds (0 to 18 m/sec) were simulated using a fan, and spray was generated using overhead sprayers above canker-infected plants (2). For dispersal studies, panel and funnel samplers collected rain splash down wind and under plants, respectively. For infection studies, grapefruit seedlings were sprayed with *Xac* inoculum ($4.7x10^5$ bacteria/ml) at 0, 4, 7 and 15 m/sec wind speeds. Volume collected, bacteria colony counts, and incidence and severity of infection were related to wind speed using regression analysis and general linear modeling.

Figure 1. Wind speed x distance effect on concentration (cfu/ml) of Xac dispersed downwind from canker infected grapefruit. NOTE: fan wind source in still air results in wind speed gradient (standard errors indicated).



The concentration of bacteria collected under trees did not change significantly at different wind speeds (P=0.05), and was consistently greater than that collected 1 m down wind at all wind speeds. However, the concentration (and total number) of bacteria collected 1 m down wind had a linear relationship with wind speed. In one experiment the total number of *Xac* bacteria sampled at canopy height 1 m down wind with no wind was 236, and at 18 ms^{-1} (~40 mph) was $2.8x10^6$, illustrating how wind increases dispersal of *Xac* in splash from a citrus canopy. The plume of dispersed canker downwind showed that as wind speed increased the plume of bacteria was dispersed further (Figure 1), despite a steep gradient in wind speed with distance from the fan. Greater quantities of *Xac* bacteria were also dispersed at greater heights.

Figure 1. Wind speed effect on %leaves infected and lesion no. per plant on grapefruit seedlings subsequent to a 2-min spray inoculation with *Xac* bacteria (standard errors indicated).



Higher wind speed also increased infection. The incidence (% leaves) and severity (lesions/plant) of infection was increased (Figure 2), with incidence two times greater at 15 m/sec (~35 mph) compared to no wind.

Storm events with high winds are a common occurrence in Florida and not only increase dispersal of *Xac* bacteria, but also cause greater rates of infection. We are currently extending the scope of these studies in natural wind/rain events and in a wind tunnel/rain generating facility at the USDA.

Citations

1. Gottwald TR, Sun X, Riley T, Graham JH, Ferrandino F, Taylor EL. 2002. Geo-referenced spatiotemporal analysis of the urban citrus canker epidemic in Florida. *Phytopathology* **92:**361-377.

2. Parker PE, Bock CH, Gottwald TR. 2003. Techniques to sample citrus canker bacteria (*Xanthomonas axonopodis* pv. *citri*) in wind-blown spray. *Plant Disease*: *In Press.*

C-5 Post-hurricane analysis of citrus canker spread and progress towards the development of a predictive model for future weather related spread

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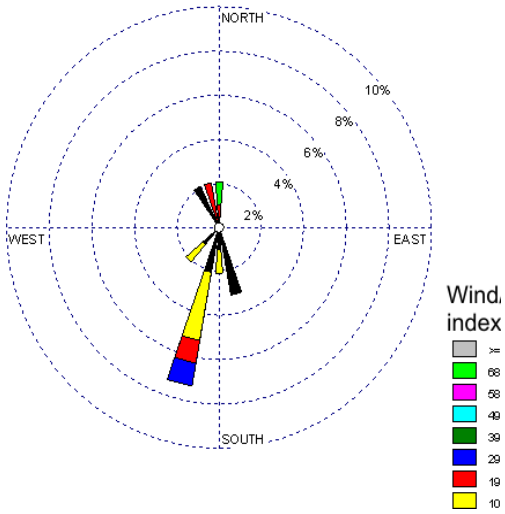
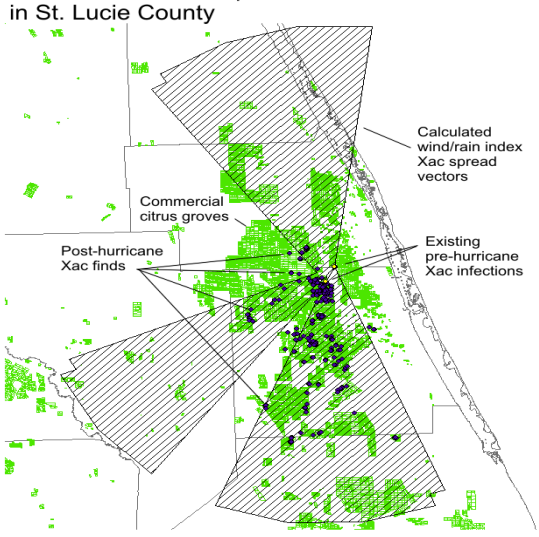
Citrus canker, caused by *Xanthomonas axonopodis pv. citri* (Xac), has been introduced into the state of Florida multiple times since the early 1900's. With each discovery, an eradication program has been put into place to eliminate the disease. The most recent program began in 1996 and is still in progress. The current citrus canker eradication program (CCEP) is a joint project operated by the Florida Department of Agriculture and Consumer Services (FDACS) and the Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA-APHIS). FDACS is responsible for the survey and eradication of Xac in the commercial industry and USDA-APHIS has largely been responsible for residential and commercial grove sentinel surveys. As Xac infections are found by either survey agency, the location (latitude and longitude) of all finds are recorded and entered into a spatial database.

Figure 1. Windrose diagram showing direction, relative intensity, and duration of wind/rain index vectors during hurricanes Frances and Jeanne in St. Lucie County, Florida during 2004.



During 2004, the state of Florida was directly affected by four hurricanes that carved different paths across the state and directly impacted commercial citrus and various residential communities with pre-existing Xac infections. In the 12 months following the 2004 hurricanes, the monthly frequency of detection of citrus canker has increased up to 4-fold, presumably as a result of wind-blown rain associated with the hurricanes. Analysis of the spatial distribution of post-hurricane Xac finds in conjunction with weather data collected during the storm events has lead to the development of model that accounts for a significant portion of the post-hurricane Xac spread. The model incorporates wind speed, wind direction, and precipitation over time to calculate wind/rain vectors (Figure 1) that predict the direction of weather-related disease spread (Figure 2). Due to the paucity of weather recording stations within the commercial citrus industry, the use of interpolated weather data derived from mesoscale weather models is being investigated as a source of environmental data to seed the spread model. Work is also underway to refine the model to predict distance of spread as well the direction of spread. Once fully validated, the model will be turned over to the CCEP and used with real-time weather data to predict Xac spread events in order to maximize the resources allocated to the eradication program.

Figure 2. Calculated wind/rain Xac spread vectors in relation to post-hurricane Xac finds in St. Lucie County



C-6 Effects of Injuries Caused by Different Instars of *Phyllocnistis citrella* on Infection by *Xanthomonas axonopodis* pv. *citri*

Belasque Júnior, J.[1], Jesus Júnior, W.C.[1], Christiano, R.S.C.[2], Amorim, L.[2], Bergamin Filho, A.[2], and Parra, J.R.P.[2]

[1]Fundecitrus, Araraquara, Brazil; [2]ESALQ/USP, Piracicaba, Brazil

After the citrus leafminer *(Phyllocnistis citrella)* was first observed in São Paulo, in 1996, an increase in the number of new plants infected by *Xanthomonas axonopodis* pv. *citri* (Xac) was observed. The presence of the leafminer altered the spatial pattern of citrus canker: less aggregated patterns and the more common presence of satellite foci, farther from the initial foci of the disease, started to appear. The spread of Xac by leafminer has no epidemiological significance. The interaction between both organisms has already been known, but there is no clear explanation for the increase observed in citrus canker incidence and severity when it is associated with the insect. Considering these facts, different experiments were carried out in controlled conditions to quantify the effect of leafminer on canker monocyclic parameters.

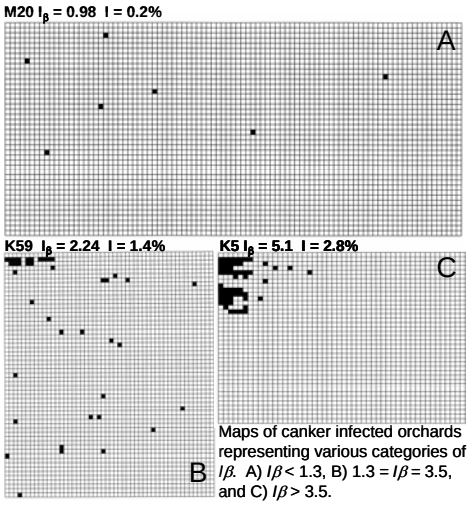
The presence of wounds in citrus plants played a major role in the infection by Xac. Only plants infested with second-instar larvae presented 100% of disease incidence up to 10 days after inoculation. Maximum incidence on mechanically-wounded plants was 80%, observed 11 days after inoculation. In the absence of wounds, only 10% of the plants showed symptoms of the disease, and the inoculated plants infested with insect eggs presented at the most 60% incidence of diseased plants 11 days after inoculation. The mean incubation period varied from 6.8 to 8.7 days, and only the treatment with egg-infested plants differed significantly from the other treatments (no injuries excluded). Plants infested with third-instar larvae were those presenting the symptoms in the shortest time period. The highest severity was observed in plants infested with third- and fourth-instar larvae. The lowest disease severities were found in plants infested with first-instar larvae, eggs and those which had mechanical wounds (no injuries excluded). Considering the age of wounds, the highest incidence of diseased plants occurred in treatments consisting of plants inoculated up to six days after the expected day for larva hatching (DEL) or on the same day of the mechanical wounds. Inoculations in plants up to 12 DEL resulted in disease symptoms, indicating the occurrence of infection approximately six days after reaching the pupae stage, when new wounds no longer occur. Plants mechanically wounded four and five days prior to inoculation with Xac did not present symptom.

We conclude: a) injuries caused by leafminer increase the incidence of the disease; b) plants infested by leafminer show disease symptoms more rapidly, as compared to the infections occurring in the absence of wounds; the reduction in the incubation period allows a larger number of disease cycles, with relevant epidemiological consequences; c) diseased tissues present higher severity and, consequently, larger bacterial populations; d) injuries caused by the insect take longer to heal and allow for infections for longer periods (several days) compared to mechanical wounds.

The experimental results presented herein refer to the changes occurred in the monocyclic components of the disease. The consequences of such changes in the time and spatial patterns of the disease still need to be investigated further.

C-7 Spatial Pattern Analysis of Citrus Canker Infected Plantings in Sao Paulo Brazil and Implication of the Asian Leafminer on Potential Dispersal Processes

T. R. Gottwald[1], R. B. Bassanezi [2], L. Amorim[3] and A. Bergamin-Filho[3]. [1]USDA, Agricultural Research Service, Ft. Pierce, FL, [2]Fundecitrus, Araraquara, SP, Brazil; [3]Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brazil.



Maps of canker infected orchards representing various categories of $I\beta$. A) $I\beta < 1.3$, B) $1.3 = I\beta = 3.5$, and C) $I\beta > 3.5$.

Eradication of Asiatic Citrus Canker (ACC) has become increasingly difficult over the last decade following the introduction of the Asian leafminer into Brazil and Florida. This prompted epidemiological studies in both countries that resulted in changes in the eradication protocols. The objective of this study was to characterize the spatial patterns of ACC in commercial citrus plantings in Brazil, to improve understanding of disease dynamics post introduction of the leafminer. The spatial patterns of ACC were mapped in 326 commercial citrus plantings, and statistically assessed at various spatial dimensions. The presence of 'within-group' aggregation in each plot was examined via beta-binomial analysis for groups of trees parsed into 3X3 quadrats. The relative intensity of aggregation was expressed as a Beta-binomial index of dispersion (I_β) and heterogeneity among plots expressed as the intracluster correlation coefficient, ρ. The population of data sets was found to fall in to three I_β categories, $I_\beta < 1.3$, $1.3 \leq I_\beta \leq 3.5$, and $I_\beta > 3.5$. These categories were related to other spatial characteristics. The binary form of Taylor's power law was used to assess the overdispersion of disease across plots and was highly significant. When the overall population of plots was parsed into I_β categories, the Taylor's R^2 improved in all cases. Although these methods assess aggregation well, they do not give information on the number of foci or aggregations within each plot. Therefore the number of foci/1000 trees was quantified and found to relate directly to the I_β categories. The $I_\beta < 1.3$ category could be explained by a linear relationship of the number of foci to disease incidence, whereas the $1.3 \leq I_\beta \leq 3.5$, and $I_\beta > 3.5$ categories were most easily explained by a generalized beta function. Spatial autocorrelation was used to examine the spatial relationships 'among groups' composed of 3X3 quadrats and determine common distances between these groups of ACC-infected trees. Aggregation was found in >84% of cases at this spatial level and there was a direct relationship between increasing I_β category and increasing core cluster size and aggregation at the among-group spatial hierarchy was generally stronger for the within-row than for the across-row orientation. Clusters of disease were estimated to average between 18 and 33 tree spaces apart, and the presence of multiple foci of infection was common. The effectiveness of the eradication protocol for removing all 'exposed' trees within 30 m surrounding each 'ACC-infected tree' was examined, and the distance of subsequent infected trees beyond this 30-m zone from the original focal infected tree was measured for each plot. A frequency distribution was compiled over all plots to describe the distance needed to circumscribe all of these outliers as a theoretical alternative to the 30-m eradication protocol. The frequency distribution was described by a monomolecular model ($r^2 = 0.98$) and used to determine that 90, 95, and 99% of all new infected trees occurred within 296, 396, and 623 m of prior infected trees in commercial citrus plantings. These distances are very similar to those previously reported for ACC exposure in residential settings in Florida.

C-8 Distance of Spread of Asian Citrus Canker from Source of Infection in Commercial Plantings in São Paulo, Brazil

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[1]ESALQ, Universidade de São Paulo Brazil, [2]Fundecitrus, Brazil, [3]USDA, Ft. Pierce, FL.

Xanthomonas axonopodis pv. *citri* (*Xac*)-infected trees continued to occur in São Paulo commercial plantings previously subjected to eradication, indicating that removing all trees within a 30-m radius of known *Xac*-infected trees was insufficient to eradicate the disease within infected orchards. Post-eradication maps of *Xac*-infected and non-infected trees were collected from 98 commercial plantings where citrus canker reoccurred following tree removal. Those maps were interrogated to determine the distance from previously identified and removed infected trees and new *Xac*-infected trees beyond the 30-m radius of destruction used for eradication.
The citrus canker-infected tree gradient decreased over distance and was well described by an inverse power law function, $y = 31413.5x^{-1.186}$, $r^2 = 0.983$. The calculated cumulative distribution of the frequency of counts of *Xac*-infected plants over distance was described well by the monomolecular model, $y = (1 - (0.79)*\exp(-(0.007)*x)$, $r^2 = 0.999$ (Fig. 1A,B). Using this model, the distances necessary to circumscribe 90, 95, and 99 percent of the new diseased trees that occurred were 296, 396, and 623 m, respectively. These calculated distances were remarkably similar to distances found necessary to remove the same proportions of the population in residential areas in south Florida: 213 m (range 152-61 3m) , 335 m (range 213-773 m), and 594 m (range 427-899 m) for 90, 95, and 99 percent captures, respectfully (Gottwald et al., Phytopathology 92:361-377, 2002). This indicates that the same spatial processes that affected the spread of *Xac* in Florida likely affected the pathogen's spread in Brazil as well.

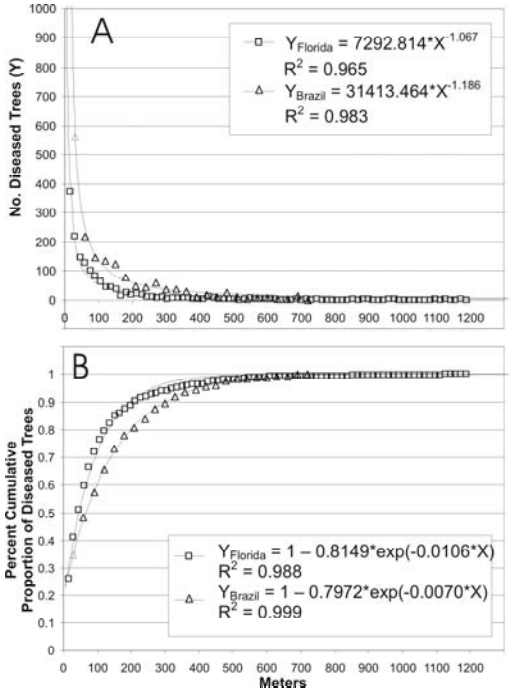

Figure 1. Disease gradients and frequency distributions of occurrence of new infections of citrus canker in Brazil and Florida. A) Disease gradients fit to an Inverse Power Law model B) Cumulative frequency distributions of the proportion of diseased trees by distance.

C-9 Use of the Green Fluorescent Protein (Gfp) to study survival of *Xanthomonas axonopodis* on citrus plant surfaces

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Although much research has been conducted on survival of *Xanthomonas axonopodis* pv. citri (Xac) as an epiphyte some important issues remain unresolved. Xac can survive on non-symptomatic leaves for short periods (hours) as an epiphyte depending on the relative humidity conditions the leaves are subjected to under drying conditions (Graham et al., 2004). This capability for short-term survival is not considered a serious risk for Xac to initiate new infections. However, previous studies of population dynamics on symptomatic or non-symptomatic plants were based on bacterial isolation on culture media, and therefore only viable and culturable cells were detected. Viable but non-culturable (VBNC) is a physiological condition described for many bacterial species and for some plant pathogenic bacteria including *Xanthomonas*. The epidemiological significance of VBNC is unknown because accurate identification of viable bacteria in planta is problematic. Transformation of the bacterium with the green fluorescent protein gene (*gfp)* gene can be used as the reporter system to locate the bacterium in or on the plant surface in situ by fluorescence and confocal microscopy. The plasmid transformants of *X. axonopodis* pv. citrumelo with *gfp* maintain virulence as compared with wild type strain. However, the GFP marker is too stable to monitor bacterial survival because dead bacterial cells still fluoresce. Instead an unstable *gfp* tranformant of *X. axonopodis* pv. citrumelo has been constructed to demonstrate the survival of this bacterium. The unstable Gfp variant is produced by the addition of an amino acid tail to the 3' terminal of the *gfp* sequence to render the protein susceptible to proteases within the bacterium (Andersen *et al.*, 1998). Hence, the living bacterium synthesizes and degrades the Gfp protein simultaneously and only cells that actively regenerate the gene product exhibit fluorescence. *X. axonopodis* pv. citrumelo isolates transformed with native and labile Gfp were sprayed on or infiltrated into *Swingle citrumelo* leaves. Bacteria survive on leaf surface when applied with a phosphate buffer osmoregulant, but die faster under low humidity conditions when applied to the leaf surface with rainwater only. Bacterial aggregation is greater in the presence of the phosphate buffer. The osmoticum appears to be responsible for the maintenance of humidity around the aggregated colonies on the leaf surface. *In planta*, bacteria remain viable when located in the intercellular space proximal to the stomatal chamber.

The *gfp* system developed with *X. axonopodis* pv. citrumelo serves as a model for further studies of *X. axonopodis* pv. citri survival *in planta* and on the surfaces of citrus fruits under environmental conditions relevant to field and post harvest conditions, respectively.

Andersen, J. B., Sternberg, C., Poulsen, L. K., Bjorn, S. P., Givskov, M. & Molin, S. (1998). New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. *Appl Environ Microbiol* **64**, 2240-2246.

Graham, J. H., Gottwald, T. R., Cubero, J. & Achor, D. S. (2004). *Xanthomonas axonopodis* pv. citri: factors affecting successful eradication of citrus canker. *Molecular Plant Pathology* **5**, 1-15.

C10 - Rationale, Techniques and Efficacy of Sanitation in Canker Eradication

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A thorough understanding of the citrus canker life cycle coupled with the typical Florida citrus production system permits optimal deployment of sanitation efforts to limit the spread of the disease by production practices. Several points will be emphasized in this presentation:

1. Public relations efforts to understand the big picture of *why* sanitation is advised and required makes compliance more likely, even to the point of making sanitation a voluntary profitable habit in all phases of production.
2. Sanitation is not necessary or advisable at *all* steps of citrus production. When necessary and barring deliberate subterfuge, complete sanitation (sterilization) is neither practical nor necessary to contain citrus canker disease based on our current understanding of the disease.
3. Points during production where sanitation is vital are identified by way of risk analysis and assessment. Three guiding principles of canker risk assessment are: a) learn the location of risks; b) minimize exposure to those risks; and c) use a sanitizer where risks are unavoidable.
4. Different sanitation methods and products have accompanying advantages, disadvantages and consequences depending on particular circumstances. Each situation merits individual analysis.

http://www.doacs.state.fl.us/pi/canker/pdf/decontamination.pdf

C-11 Standardization of diagnosis for citrus bacterial canker: the EPPO protocol and its use for detection in citrus fruits.

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The European and Mediterranean Plant Protection Organization (EPPO) develops diagnostic protocols to provide standardized schemes for detection and diagnosis of a broad range of organisms harmful to plants.

Different methods for detection and identification of *Xanthomonas axonopodis* pv. *citri*, causal agent of citrus bacterial canker (CBC), have been reviewed and those with the greatest potential, were selected and optimised in order to set up a diagnostic protocol for routine analysis. The integrated approach of diagnostic includes several methods for the detection of all of the CBC strains and types in symptomatic or symptomless plant material. The selected techniques and probes are: a) isolation on different media (NGA, YPGA, KCB), b) ELISA, using specific monoclonal antibodies, c) PCR using primers 2 and 3 designed by Hartung et al (1993) and J-pth1 and J-pth2 designed by Cubero and Graham (2002) and Real Time PCR (Mavrodieva et al 2004, Cubero and Graham, 2005) and d) bioassay on detached or attached leaves, or on *in vitro* growing plants, followed by isolation. All the methods gave reliable results for the detection of the bacteria in symptomatic plant material. PCR seems to be the most appropriate for a rapid screening test, even though the use of two different PCR primers and protocols is advised, in order to increase the accuracy of the diagnosis. They can be also used for detection of the bacterium in asymptomatic plant material. In addition, bacterial isolation and pathogenicity tests are required to demonstrate the viability of the pathogen from symptomatic or symptomless samples. The identification and characterization of the CBC bacterium can also be achieved by pathogenicity tests, metabolic profiling, fatty acid analysis, ELISA, BOX and ERIC PCR and other techniques. All this methodology will be published shortly as the EPPO standard for diagnosis of CBC.

This EPPO diagnosis scheme has been successfully applied during the last three years in the analysis of citrus fruits showing symptoms similar to those of CBC imported in Spain from countries where *X. axonopodis* pv. *citri* is present.

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C 12 Epidemiology, management and effect on yield of citrus canker under endemic conditions in Paraná, Brazil

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Citrus canker caused by *Xanthomonas axonopodis* pv. *citri* was first reported in Brazil in the western region of São Paulo state. Whereas in São Paulo eradication is mandatory, in Paraná state it is not. This fact allowed us to investigate the epidemiology of citrus canker under endemic conditions in Paraná as affected by different control measures (windbreak and copper spray) from December 2002 to April 2005. The experiment was carried out in a commercial orchard located at Ourizona, western Paraná, about 100 km south of São Paulo state. Cultivar used was Pêra on Rangpur lime, aged two years at the beginning of the experiment. Windbreaks consisted of a screen (70% porosity) supported by wood stakes 3 m high. Copper oxychloride was applied monthly, except during the period May-August each year. All treatments showed a fast progress of infection around November-January (wet season) followed by a marked decline after that. Copper spray, windbreak, or combination of the two methods did not completely suppress the disease (Figure 1). Epidemic was more severe in 2003-2004 than in 2004-2005, which corresponded well to the levels of leaf miner in each period (peaks of 60 and 12% of infested leaves, respectively).

Despite poor control, copper sprays allowed significant (P<5%) higher yields in 2003-2004 (28.7 versus 13.7 kg/plant in plots with windbreak and 30.4 versus 13.8 kg/plant in plots without windbreak). No differences in yield were detected in 2004-2005 (76.4 versus 72.1 kg/plant with windbreak and 69.1 versus 79.9 kg/plant without windbreak). Contrary to data published in literature, effect of windbreaks in both disease progress and yield was not detected.

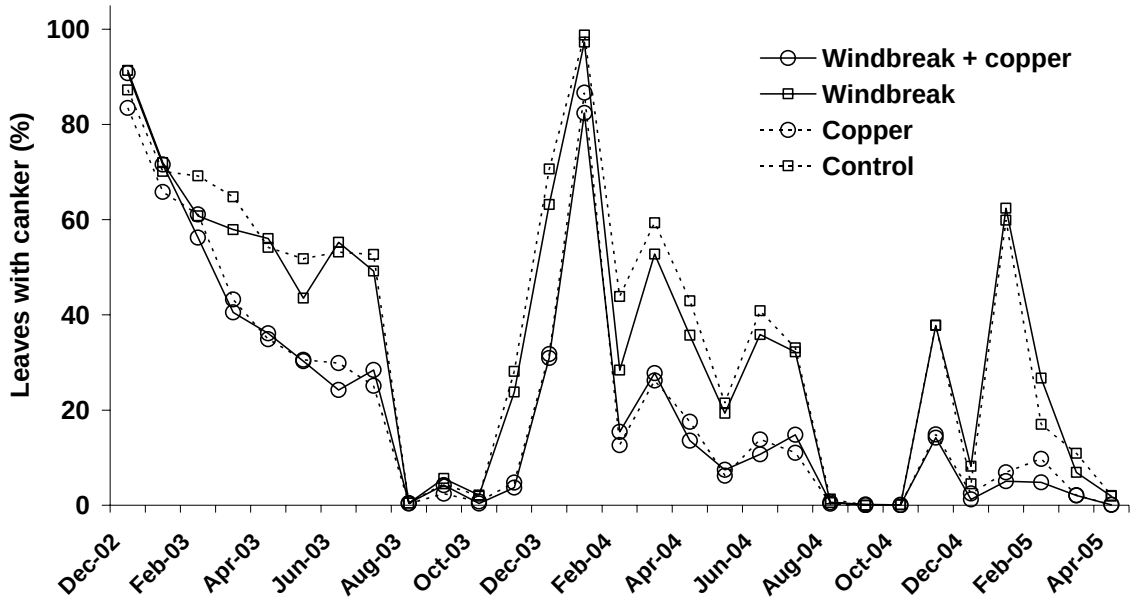

Figure 1. Disease progress curve (% of diseased leaves) of citrus canker under different treatments in endemic conditions (Paraná, Brazil).

C -13 Integrated Management of Citrus Canker in Southern Brazil

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Citrus canker caused by *Xanthomonas axonopodis* pv. citri (=*Xanthomonas campestris* pv. citri) is a serious bacterial disease for citrus production in many areas around the world. Since the first introduction in the western region of São Paulo in 1957, the disease has become an important problem in several states of Brazil. Citrus canker has been reported in the North, Central and Southern regions of Brazil, including Rio Grande do Sul, Santa Catarina, Paraná, Mato Grosso do Sul, Mato Grosso, Goiás, São Paulo, Minas Gerais and Roraima. In 1999, a major eradication campaign was started in the main citrus area of São Paulo state to eliminate the disease from more than 4,000 citrus blocks. Besides that, the introduction of the Asian leafminer (*Phyllocnistis citrella*) greatly increased the severity of citrus canker in Brazil. Therefore, an active disease suppression program has been practiced with the adoption of exclusion, sanitation, eradication and control measures in an integrated program in the major Brazilian areas of citrus production. In regions free of canker, exclusion measures have been adopted to prevent introduction of the disease. In regions where the disease has been endemic, a management program has been implemented for prevention and control of citrus canker. A major component of this approach is discouraging the planting of highly susceptible citrus cultivars such as grapefruit, Mexican lime, Navel and Hamlin oranges, Murcott tangor and Orlando tangelo. In contrast, the growers are encouraged to plant citrus cultivars more resistant to canker selected from screening program, such as Cadenera, Folha Murcha IAPAR-73, Jaffa, Pera, Salustiana, Shamouti, and Valencia oranges, Montenegrina, Ponkan, Satsuma and Willowleaf mandarins, and Tahiti lime. Production of nursery trees in exclusionary screen houses has been an important measure to obtain citrus trees free of canker. Sprays of copper based bactericides and use of windbreaks are also recommended to suppress the disease and prevent the spread of the bacterium to new areas. Control of the Asian leafminer in new orchards is also used to prevent the interaction with bacterial infection and consequent inoculum production. Research programs on citrus canker have also been implemented in Brazil to better understand different aspects of the disease and to develop more appropriated control measures. Studies on the genetic diversity of strains of *X. axonopodis* pv. *citri* has demonstrated a clonal population structure of the bacterium in Brazil. Epidemiological studies provide a better understanding of disease progress under our conditions and in the presence of Asian leafminer. Screening of citrus germplasm under field conditions has identified cultivars less susceptible to canker and a late Valencia (Folha murcha) has been widely adopted for new citrus plantings. Development of transgenic Pera sweet orange with resistance to canker has been investigated using the Sarcotoxin IA as the transgene. Field trials of chemicals for control of canker have evaluated Induced Systemic Resistance compounds (ISRs) and other novel chemicals in combination with copper formulations with the aim to reduce the rate of copper usage.

C-14 Citrus Canker Sanitation in Groves by Drastic Pruning

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The current method of control of citrus canker in São Paulo State, Brazil, is the eradication of symptomatic plants and suspected of infection plants. Because of this, there are great economical losses to the citrus growers when infected groves are found. Groves with more than 0.5% of infected plants are completely eliminated, and incidences equal or smaller than 0.5% determines an eradication in 30 meters of radius. Quarantine restrictions are applied to eradicated areas and new citrus plantations are prohibited up to two years after eradication. So, any methodology that eliminates the pathogen and implicates in a smaller number of eradicated trees or economical losses has a great potential of use. In an area with long history of citrus canker infection, 10,955 plants were pruned in 17 contaminated groves. The incidence of diseased plants before pruning varied between 0.09 and 100%. Two methodologies were used to decide the number of pruned plants in each grove. In the first experiment (five groves), all diseased plants were eliminated, and when two or more diseased plants were adjacent, the surrounding plants in a 30 meters radius from the diseased plant were drastic pruned. In the second one (12 groves), any diseased plant wasn't eliminated and all the plants were pruned in the cases with more than 0.5% of incidence. In smaller incidences ($\leq$0.5%) the infected plant and the surrounding plants in a 30 meters radius were drastic pruned. The drastic pruning consisted of completely elimination of all branches, leaves and fruits, including dropped leaves and fruits. These vegetal material were collected and burned and the soil were buried. After pruning, the remaining trunk with three main branches were painting with lime (25%) and copper oxichloride (1%). All citrus trees were inspected in a 2-km radius from each pruned grove, in a maximum frequency of 3 months. The diseased plants found during these inspections were eliminated. In 6 pruned groves (35.3%), one of the first experiment and five of the second one, diseased plants were found between 8 and 11 months after pruning. The initial incidence in these re-infected groves were 7.04, 2.63, 4.39, 49.68, 100.00, and 0.87% of infected trees, and the incidence found after pruning were 0.43, 0.12, 0.75, 0.54, 31.06, and 0.62%, respectively. In the other eleven groves (64.7%) infected plants were not found after two years of pruning. The pruned plants returned to produce fruits after two years. Considering these results, the drastic pruning could be applied as a method of sanitation in endemic situations of citrus canker, but not in eradication or suppression programs, like the one applied in São Paulo State at the moment.

C-15 CHEMICAL CONTROL OF CITRUS CANKER IN LEMONS (*Citrus limon*, (L.)Burm. f) IN TUCUMAN, ARGENTINA

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Tucuman province in Argentina is located in the northwest of the country (26º S. lat.) and is the leading producer of lemons with 1.200.000 tons (90%). Nearly 340.000 tons (30%) goes for fresh fruit to the international markets, which is 97% of the total lemon fresh fruit exports from Argentina. The citrus area in Tucuman has fertile soils, hot and humid summers with a rainy season from October to April (annual rainfall ranges from 800 to 1500 mm). Under these conditions lemon trees are vigorous and high yielding with an expanded flowering season resulting in production of fruits of different sizes and ages on the tree at the same time during the growing season. Under these conditions citrus leafminer affects as much as 80% of summer flush from December to March, and chemical control of the insect and citrus canker is necessary to produce export quality fruit. Citrus canker, caused by *Xanthomonas axonopodis* pv *citri*), has been present in Tucuman since 2002, but is still unevenly distributed in the province. Severity and incidence of canker in the affected areas varies widely, mainly based on annual rainfall.

Spray trials were conducted to evaluate the efficacy of different bactericides and the interaction with leafminer on control of citrus canker. These trials began in 2002 in an irrigated orchard of Limoneira 8 A Lisbon lemon on Swingle citrumelo rootstock planted in 1997. Bactericide applications were made with a conventional high volume speed sprayer at a rate of 25 l/tree. Bactericides tested were copper formulations including 5 treatments with copper hydroxide, 13 treatments with copper oxychloride in two doses, alone and in combination with mancozeb, and with and without the use of spray oil as an adjuvant. The effect of leaf miner control was studied with applications of the insecticide, abamectine (0.02%) in combination with copper oxychloride plus mancozeb and compared with insecticide alone. The treatments commenced at petal fall (end of September) and a total of six applications were made at 28-day intervals from September to February each year. A non-sprayed check treatment was included in all trials. The trial layout was a randomized block design and each block consisted of three rows of 20 trees replicated four times.

The results confirm: 1) Copper bactericides effectively control citrus canker on fruits and leaf flushes; 2) Copper hydroxide treatments produce the highest percentage of fruit free of canker (clean fruit); 3) Copper oxychloride at higher doses (0.3%) achieves a higher percentage of clean fruit than lower doses (0.2%) but produces less marketable fruit due to phytotoxicity; 4) Spray oil does not improve canker control with copper oxychloride but increases the percentage of marketable fruit; 5) Addition of mancozeb with copper oxychloride also does not improve canker control; 6) The percentage of clean fruit is not increased as a result of leaf miner control; 7) However, leafminer control alone produces 30% more canker free fruit when compared with non sprayed check treatment.

Citrus canker control was more effective in years with dry springs and/or summers. During the 3 years of trials an average of 80% fruit free of canker was obtained. Percentage clean fruit in years with rainfall below or similar to the average of the 30-year rainfall were 97% and 75%, respectively.

C-16 Control of Asiatic Citrus Canker and Citrus Bacterial Spot with Bacteriophages in Florida

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Asiatic citrus canker, caused by X. axonopodis pv. citri (*Xac*), is a serious threat to Florida's $8.5 billion citrus industry. The pathogen was introduced to Florida several times during the 1990's, and the disease is currently under eradication. However, recent hurricane events hindered the eradication efforts and distributed the pathogen over wide areas. Several control measures of the disease are under development including the use of bacteriophages. A collection of 81 phages was established from the following sources: 13 *Xac* phages from existing collections, 4 phages of other Xanthomonads that are also pathogenic on *Xac*, and 64 phages isolated from diseased plant tissue in Florida and Argentina. In greenhouse experiments a single foliar application of a mixture of four phages (2×10^8 PFU/ml) significantly reduced the disease severity caused by a "Miami" strain of *Xac* (10^6 PFU/ml). Field trials were conducted with citrus bacterial spot (CBS), incited by *Xanthomonas axonopodis* pv. *citrumelo* (*Xacm*), since the current regulations do not allow field research with citrus canker in Florida. In trials conducted in a commercial citrus nursery, twice-weekly application of a mixture of three phages at 2×10^8 PFU/ml significantly reduced the CBS disease severity.

C-17 Economic Analysis of Florida's Citrus Canker Eradication Program

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Abstract: The rapid expansion and integration of international trade, increased tourism, and changes in methods of production in recent decades have increased the likelihood of the introduction of invasive species to U.S. agriculture. Invasive species can have adverse environmental and/or economic impacts when introduced to a region. Economic impacts include marketing, production, and trade implications.

One such invasive species imposing adverse economic impacts to the Florida citrus industry is a bacterial disease known as citrus canker (*Xanthomonas axanopodis* pv. *citri*). Citrus canker causes lesions on the leaves, stems, and fruit of citrus trees. The disease adversely affects the proportion of fruit intended for the fresh market, serves to weaken citrus trees, leads to a reduction in yields, and leads to higher costs of production.

The current effort to eradicate citrus canker from the industry, the Citrus Canker Eradication Program (CCEP), has been mired in controversy associated with public opinion and legal action. An economic/benefit-cost analysis was conducted to determine if the CCEP is, indeed, a useful policy tool in combating the economic ramifications associated with citrus canker.

In this paper, an economic analysis of the Citrus Canker Eradication Program on the Florida citrus industry is characterized through employment of a benefit-cost analysis of retaining the current policy. A benefit-cost analysis of the Citrus Canker Eradication Program (CCEP) in Florida was developed using the predicted values of the benefits and the costs associated with the policy. The actual expenditures of implementation through 2004 are weighed against the projected loss of revenue and the cost savings associated with an industry with pervasive citrus canker in an attempt to assess the net benefits of the policy. Three segments of Florida's citrus industry were analyzed separately: 1) processed oranges; 2) fresh and processed grapefruit; and specialty citrus fruit.

C-18 Implementation of the Citrus Canker Eradication Program: Lessons Learned from the Stop-and-Go approach to Eradicating Exposed Trees

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Abstract: The spread of citrus canker in Florida in the late 1990s led to an intense citrus canker regulatory program of eradication. However, the Citrus Canker Eradication Program (CCEP) was mired in controversy associated with public opinion and legal action from its onset. In October 2000, a Broward County court ruling stated that the state failed to follow proper rulemaking procedures when implementing eradication guidelines based upon the 1900-foot radius rule in which any tree infected with the disease, as well as all citrus trees within a 1900-foot radius, be removed. Such was the beginning of the so-called "stop-and-go" approach to the eradication effort with respect to trees *exposed* to citrus canker. During such periods in which eradication was halted, sentinel surveys were also suspended. Although it is unknown precisely how the disease spread during these suspensions, the costs associated with the eradication program were destined to increase as legal expenses were incurred and quarantined areas expanded.
This presentation explores the additional costs to the CCEP associated with the stop-and-go approach to eradicating citrus trees exposed to citrus canker, which include legal fees and additional compensation to producers. Prior to the injunctions placed upon tree removals, the deputy commissioner of agriculture, Craig Meyer, fully anticipated containment of the disease by late January 2001. A benefit-cost analysis of the CCEP was conducted under the assumption that eradication efforts would have been successful by the end of 2000 as predicted by officials. The procedures outlined in the CCEP would require the continuation of sentinel surveys for an additional two years after the last positive citrus canker find. Under these circumstances, it is expected that the eradication program will continue on until January 2003. The estimated benefit-cost analysis of proceeding with the CCEP in the absence of the "stop-and-go" approach to eradicating exposed citrus trees, assuming a discount rate of 10%, yielded an estimated increase in discounted net benefits by more than $216 million over time. The discounted costs associated with the CCEP decline by 45% to from $367 million to $200 million in the absence of the stop-and-go scenario. The discounted net benefits of the program proved to be even more persuasive of the effectiveness of the CCEP without the "stop-and-go" approach to eradication.

The results of this study will be useful when considering the eradication policy of Huanglongbing (Citrus Greening) due to the very nature of the disease and the economic implications associated with an HLB endemic citrus industry. The economic implications associated with established HLB to Florida include additional costs to production, loss of market access, yield reduction industry-wide, and higher prices. The results of this analysis suggest that swift action is the best policy when eradication is the only method controlling the establishment of a destructive pest to citrus groves. In the case of HLB, it is best to eradicate as quickly as possible to minimize the costs to the state, federal government, and to the industry.

C-19 Real Time PCR and Allelic discrimination to detect, enumerate and differentiate *Xanthomona axonopodis* strains causing citrus canker

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Wide host range A strains of *Xanthomonas axonopodis* pv. citri are able to infect almost all citrus species, however restricted host range A*and A^w strains affecting only Mexican lime and *Citrus macrophylla*, occur in Southwest Asia (A*) (Verniere *et al.*, 1998) and similar strains have recently been found in South Florida (A^w) (Sun *et al.*, 2004). Citrus canker strain types A, B and C may be discriminated by BOX and ERIC PCR primers and within A type this analysis also differentiates restricted host range strains from wide host range strains (Cubero and Graham, 2002). The main difference between profiles obtained by ERIC PCR for these two groups strains is due to a fragment, that corresponds to the leucine responsive regulatory protein gene (*lrp*) (Cubero and Graham, 2004). The fragment is present in A strains but not in A* or A^w. Absense of this fragment is due to the small variation in nucleotide sequence that affects its amplification by ERIC-PCR primers.

A small sequence difference between restricted and wide host range strains of *X. axonopodis* pv. citri was employed to design an allelic discrimination assay. Two TaqMan MGB probes labelled with FAM and VIC were designed. These probes are based on a unique nucleotide in strains A vs. A*/A^w at position 417 of the *lrp* (accession no. AY227405 and AY227411). Both probes were included together in Real Time (RT)PCR reactions performed with pure bacterial cultures and leaf samples infected with the different strains. The A strain produced the most fluorescence from FAM, whereas A* or A^w produced the most fluorescence from VIC. The bacterium causing citrus bacterial spot, ‚*X. axonopodis* pv. citrumelo, yielded the least fluorescence from both dyes, allelic discrimination of strains corresponds with grouping of the strain's based on polyphasic analyses described elsewhere. Genotyping by the allelic discrimination method described was also applicable with DNA isolated from leaves infected with the different strains.

TaqMan MGB probes designed for allelic discrimination were also used for bacterial enumeration in planta and comparable to quantitative RT-PCR with probes based on *pth* and ribosomal sequences from A strain. In most of the cases, but not all, a correlation was found between bacterial numbers obtain by RT-PCR and plate counts on selective media.

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C-20 A 20-minute real-time TaqMan® assay for fast and reliable diagnostics of citrus canker.

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Citrus canker caused by the *Xanthomonas citri* (*X. citri*) bacterium continues to spread in the State of Florida despite best efforts of the Citrus Canker Eradication Program (CCEP). In July 2003 a Broward Co. judge ruled for DNA-based confirmation of all presumably citrus canker positive trees before their eradication. The FDACS implemented a SYBR® Green real-time PCR assay with *X. citri* universal primers (Mavrodieva et al, 2004). APHIS-PPQ-CPHST continues to work on improving field diagnostics for this disease.

A highly sensitive, rapid, and robust real-time TaqMan PCR for use on the Cepheid SmartCycler platform was developed and optimized by the U.S. Army's Biosensors Team laboratory in collaboration with NPGBL. TaqMan assays provide added specificity due to probe hybridization to the PCR amplicon. Twelve primer pairs were tested for specificity with all 5 known *X. citri* strains A, B, C, A* and A^W. The best candidates were forwarded for further testing with a panel of additional 26 citrus canker isolates from different geographical regions. The best performing primer pair was chosen and two Taqman probes were designed and evaluated. A 20-minute TaqMan assay was optimized on the portable Cepheid SmartCycler® instrument using the selected primers and a probe. Dynamic range of the assay was evaluated and the limit of detection (LOD) was determined to be 100fg of *X. citri* DNA. Highly reproducible results were obtained when the DNA panel of 26 citrus canker isolates was re-tested at the LOD concentration. The assay was tested with a limited number (34) of environmental samples that tested positive for citrus canker previously. A hundred percent correlation of the results was obtained. High C_T values (above 36) were obtained when healthy plant DNA extracted with the DNeasy® Plant Mini kit from Qiagen was tested. However, our preferred procedure for citrus canker testing does not involve extraction of total plant DNA. We rather select single canker lesions and crush them in buffer to release bacteria for testing. Thus we limit the amount of plant DNA in the sample that should minimize unspecific amplification in this assay. The assay was tested with total plant DNA extracted from citrus plant infected with other pathogens (HLB, CVC, P. citricola, CTV). High C_T values (above 36) were obtained probably due to the presence of plant DNA.

Currently we are working on the transferring of the assay to other real-time PCR instruments/platforms. We will test different extraction procedures and their effect on amplification. Further testing of the citrus canker TaqMan assay with environmental samples will be necessary before its deployment to the filed.

C-21 Rapid Genetic Typing of Strains of *Xanthomonas citri*, the Causal Agent of Citrus Canker in Florida

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Citrus canker is one of the most important diseases of citrus, and is caused by two phylogenetically distinct groups of *Xanthomonas* strains, one originating from Asia (*X. citri* pv. *citri* groups, including strains A, A*, and A^w) and the other from South America (*X. citri* pv. *aurantifolii* group, including strains B and C). The Wellington strain or genotype, A^w was previously described from Florida. We have developed a novel primer, DG04, that allows us to differentiate not only all major strains of *X. citri*, but also several new strains or genotypes within strain A, designated as the Miami, Manatee I and II, and West Palm Beach strains, using a simple PCR test based upon their fragment length polymorphism. Sequence analysis of the PCR products revealed their sequence variability among these strains. The discovery of these new strains/genotypes of *Xanthomonas citri* in Florida indicates the complexity of citrus canker bacteria in this state, and may imply that multiple introduction events have taken place. The host-pathogen interactions for these newly identified strains are under investigation

C-22 Detection of Manatee Genotypes of Citrus Canker Bacteria in Hillsborough Co, Florida

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Citrus canker was found in both Sun City Center and Ruskin of Hillsborough County, Florida in December, 2004. Since May of 2005, the disease has subsequently been detected in several orchards near the residential Ruskin. Thirty three citrus canker bacterial cultures obtained from door yard and commercial citrus trees in Sun City Center and Ruskin plus some representative cultures from surrounding canker sites were tested for their genotypes to determine the possible links between new canker finds and the known ones in the area. Using the DG04 primer, Manatee genotype of *Xanthomonas axonopodis* pv. *citri* was confirmed on all cultures collected from commercial citrus in Ruskin area while Miami and Manatee genotypes were detected on residential citrus trees in both Sun City Center and Ruskin. All cultures from other representative areas were positive for Miami genotype. The results from this investigation indicated that Manatee genotype of canker bacterium may have dispersed, from Manatee County where the genotype was last found in1999, to the poorly managed grapefruit orchard, Bay Breeze Grove, where over 2-year-old canker lesions were found in most trees. The disease apparently remained there since and served as an inoculum source for many years in the area.

C-23 Using PCR for detection and quantification *of Xanthomonas axonopodis* pv *citri* in wind driven splash

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An eradication program has been developed to remove citrus canker (caused by *Xanthomonas axonopodis* pv *citri*, *Xac*) from Florida (1,4). The eradication program is based on knowledge of the disease epidemiology. Additional information on the spread of *Xac* bacteria in wind blown splash will provide further insights into pathogen dynamics, and ways of detecting and quantifying epidemiologically significant (living) bacteria of *Xac*. The objective here was to assess a molecular method (PCR) and dilution plating for detecting and quantifying the pathogen in wind-driven splash.

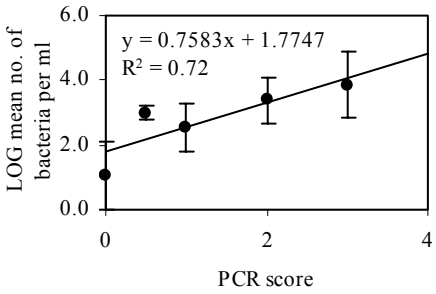
Samples of wind-driven splash (a total of 314) were collected down wind from canker-infected trees. The systems used to simulate rain splash, and the sampling procedures, have been described previously (3). Control samples comprised water and known positive samples of *Xac*. The samples were subject to dilution plating on nutrient agar and the colonies counted after 5 days and compared to that estimated using an established citrus canker SYBR Green real-time PCR method for detection from symptomatic tissues using universal primers VM3+4 (2).

Table 1. Agreement between samples of *Xac* detected by dilution plating and real-time PCR in each of the PCR categories.

PCR Category	Total	Agreed	Disagreed	Agreed (%)	Disagreed (%)
0	124	49	75	39.5	60.5
0.5	7	7	0	100.0	0.0
1	135	132	3	97.8	2.2
2	45	44	1	97.8	2.2
3	3	3	0	100.0	0.0
Total	314	235	79	74.8	25.2

Detection agreed between the two systems on 75% of the samples tested (235 of 314 samples). However, false negatives using PCR were found in 75 (24%) of samples, all of which contained 3 to 863 bacteria/per ml as detected by dilution plating, but only 4 samples (1%) were positive using PCR, but not by dilution plating. However, at PCR scores of 0.5 and above, there was excellent agreement on detection of the bacteria between the two methods. There was a positive linear relationship between the real-time PCR score and log number of living bacteria collected/ml (Table 1), although a large range of living bacteria/ml existed within each PCR category, implying some discrepancy in quantification of bacteria between the two methods.

Figure 1. Relationship between LOG mean bacteria and PCR score



No modification was made to the PCR protocol and no splash-sample concentrating procedures were performed. The results confirm previous observations on the sensitivity of real-time PCR for detecting citrus canker bacteria (down to 10^3/ml, 2). Differences existed in quantification of bacteria between the two methods. For epidemiological studies a more sensitive PCR protocol is desirable, particularly one that can quantify epidemiologically active bacteria. Development of such a system is currently underway.

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C-24 Survey Strategies Utilized in Commercial Groves and Residential Properties for Citrus Canker Detection.

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As part of the citrus canker detection program, commercial groves and residential properties are inspected utilizing a combination of 3 strategies which rely on Federal, State and Industry resources where applicable. In areas where citrus canker is not known to exist, commercial groves are inspected annually by FDACS-DPI inspectors and are augmented by both Sentinel Grove Survey and Self Survey which integrates USDA survey technicians and industry personnel respectively into a combined detection effort. Survey of residential properties is performed through the USDA Sentinel Survey program. As a means to early detection in a residential setting, a 12 x 12 grid is used to divide each square mile into 144 sub-sections and one susceptible cultivar in each grid is identified as a sentinel tree. A maximum of 144 trees per square mile are surveyed every 60 days.

Months following the devastation brought on by 3 major hurricanes that hit Florida in 2004, the incidence of new citrus canker finds grew rapidly and required the movement of staff from residential Sentinel Survey to Sentinel Grove Survey in July 2005. Two post hurricane sentinel 60-day cycles had been completed by this time. Sentinel Grove Survey divided Florida's commercial citrus production areas into 369 sub-areas each containing 36 mi^2. Three hundred sixty nine canker-free 36 mi^2 township-range sub-areas were included in this survey cycle while all known infected sub-areas were under a 5-mile delimited survey that was performed by FDACS-DPI. From within each canker-free sub-area, 2 grove blocks ranging between 20 and 50 acres containing grapefruit or early oranges were surveyed. Once a positive block was detected the sub-area was turned over to the delimiting survey crews. At the completion of the first phase, the procedure was repeated however 4 new blocks were selected per sub-area until the completion of phase II. The process would continue in phase III by adding 6 new blocks. Only negative sub-areas would be carried into the next phase. At the end of the first phase, 12 sub-areas were confirmed positive for citrus canker out of 334 surveyed. Since the start of phase II, 6 out of 317 sub-areas surveyed have been positive for citrus canker. During the months of June and July 2005, an outreach program was initiated to train industry workers to assist with survey efforts. Approximately 2000 participants completed the self-survey program in the 2 month period. Since October 2004, 300 new finds have been confirmed with a peak of 57 grove blocks being reported in the months of July. The increased number of grove acres surveyed by the combined efforts of USDA, FDACS-DPI and industry workers lead to the early detection of new outbreaks following the spring flush.

C-25 Florida Grower Self-Inspection Program for Citrus Canker

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Topic Area: Survey, Oral

Citrus canker (CC), caused by bacterium, *Xanthomonas axonopodis* pv *citri*, has been a challenging invasive disease for the State of Florida. The current eradication campaign for CC began in 1995. Increased international travel, the devastating 2004 hurricane season, legal disputes, movement of infected plant material by home owners, and spread by equipment and personnel of the commercial citrus industry, have made efforts to contain and eradicate the disease more difficult. The CC eradication program (CCEP) faced a shortage of manpower for detection and timely removal of infected and exposed trees post-2004 hurricane season. As with any agricultural protection program, industry support is an essential element. The Florida Citrus Industry has played a key role in two important areas: (1) decontamination of personnel and equipment, and (2) a self survey of citrus groves for CC symptoms to augment surveys conducted by the CCEP. These initiatives are currently being implemented as a part of the CCEP Business Plan, Schedule 27 for grove best management practices (BMPs) for CC. Timely detection of the disease is crucial to limiting the further mechanical, human and weather related spread within or to adjacent properties. The goal is to minimize the number of new CC foci and reduce the acreage of tree removal. Beginning in 2005, the CCEP Business Plan was required for each entity to qualify for a commercial grove CCEP compliance agreement. To gain compliance, all field personnel, grove foremen and crews are required to be trained each year in citrus canker decontamination by a company appointed certified training officer (CCEP Grove / Caretaker Compliance Agreement Section 1.2). Another element of CC self inspection is to develop and adopt a management plan that includes a quarterly self-survey or at least two survey cycles between May and November.

Since 2002, the University of Florida, IFAS has had a CC educational program targeting the residential sector, as well as, commercial citrus and non-citrus commercial businesses. In cooperation with state and federal regulatory agencies, survey methods and protocols were developed into a PowerPoint presentation for grove self-inspection. A series of workshops were launched to promote and distribute the self-survey materials. More than 2300 English-speaking grove workers and more than 500 Spanish-speaking workers were trained. The overall benefits of these activities have been 1) an increased statewide awareness of citrus canker biology to slow the illegal movement of plant material by residents throughout Florida, 2) increased emphasis on the importance of decontamination practices, and 3) self-survey to augment the regulatory agencies' survey efforts to increase the timeliness of disease detection and reduce the spread of citrus canker bacteria within the commercial citrus industry. CC educational activities have been carried out in twenty Florida counties and further extended for outreach to the national and international level.

C-26 Old *versus* new canker eradication strategy in São Paulo, Brazil

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Citrus canker caused by *Xanthomonas axonopodis* pv. *citri* was first reported in Brazil in 1957. Since then, eradication is mandatory in São Paulo, the main citrus producer state in the country. Until recently, citrus trees within a 30-m radius of a symptomatic tree were considered to be exposed and were removed in an attempt to achieve eradication. Despite complete eradication was never achieved, canker was kept under very low incidence until 1996. Just after the identification of the Asian leaf miner (*Phyllocnistis citrella*) in São Paulo, in 1996, canker incidence increased drastically, reaching a peak in 1999 (Fig. 1, left). Based on the fact that canker resurgence was higher than 80% in eradicated groves between 1997 and 1999, the eradication strategy was then changed (July 2nd, 1999) and now it is based on both distance for diseased trees and disease incidence, such that if disease incidence in a given block is equal to or less than 0.05, then the above eradication strategy is used. However, if disease incidence is greater than 0.05, then all trees within the infected planting or block are considered exposed and are destroyed. This new strategy showed to be efficient and the percentage of blocks known to have symptomatic trees dropped from 0.70% in 1999 to 0.11% in 2005 (Fig. 1, right). Despite that, São Paulo is far away from a complete eradication of the disease, indicating that a new, more stringent, strategy is necessary.

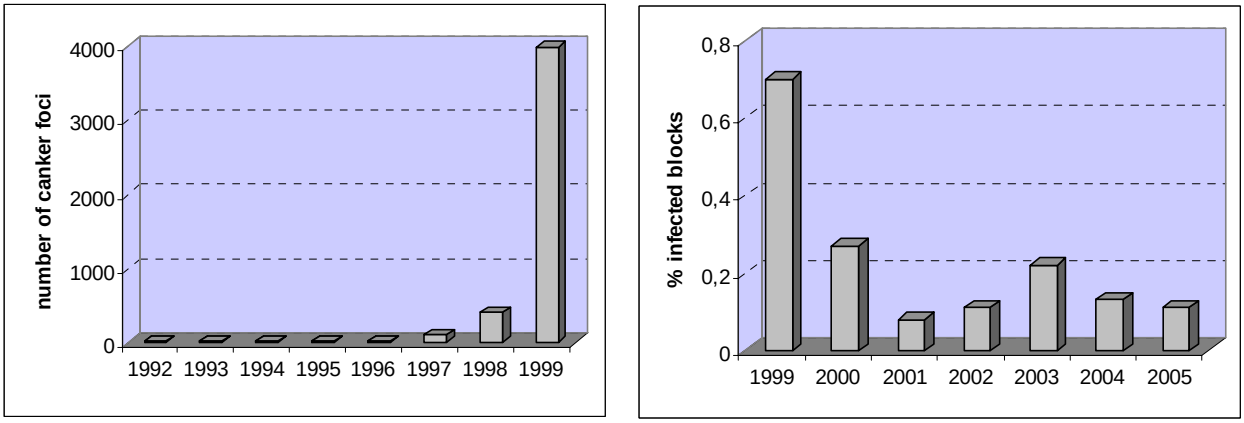

Figure 1. Left: Number of foci of symptomatic canker trees in São Paulo from 1992 to 1999 (Asian leaf miner was identified in São Paulo in 1996). Right: Percentage of blocks with symptomatic canker trees in São Paulo (new strategy of eradication introduced in 1999).

C-27 AN OVERVIEW OF THE *XANTHOMONAS AXONOPODIS* PV CITRI GENOME

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Xanthomonas axonopodis pv citri (Xac) is responsible for citrus canker disease, one of the most serious of diseases of citrus. As a member of the genus Xanthomonas, it shares basic mechanisms of pathogenicity as well as underlying genomic sequence organization with other members of the genus. The Xac genome, as well as the genome of the related pathogen *X. campestris* pv. campestris (Xcc) have been sequenced (da Silva et al., 2002. Nature 417:459-463). Comparative analyses of the genomes have identified subtle differences in the genetic complement of the two organisms that may be related to the invasive nature of Xcc as compared to the localized response of citrus to infection by Xac. Both organisms regulate pathogenicity and host range using the HRP/*avr* system. Earlier work had shown that in the case of Xac, a member of the *avr* gene family was responsible for the induction of the canker lesion phenotype characteristic of citrus canker disease. The genome of Xac is also rich in transposable elements, many of which flank regions thought to be important for virulence. These regions thus are important in the evolutionary history of the pathogen. The full genome sequence data for Xac provides, in a narrow sense, everything that there is to know about the bacterium. In a larger sense however, major questions remain, not the least of which is how to translate the available genomic knowledge into practical methods for disease prevention or control.

C-28 Identification of an avirulence gene in *Xanthomonas axonopodis* pv. *citri* strain A^w

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A new strain of the citrus canker bacterium (*Xanthomonas axonopodis* pv. *citri*) was detected in Florida. It was significant in that it was primarily pathogenic on Key lime trees, but not on grapefruit trees. This strain has been designated as Xac-A^w and is genetically similar to Asiatic citrus canker. This strain has caused concern among regulators with regard to how to treat this bacterium in the eradication program. Of major concern was the stability of the bacterium in regard to host specificity of the strain; for example, could the strain mutate to attack grapefruit and other citrus plants. We investigated the frequency of the development of mutants, the transfer of genes by conjugation, and the presence of an avirulence gene to grapefruit in the genome. We never were able to find a mutant, either natural or induced, that changed host range. In conjugation studies, transfer of marker genes on the chromosome to and from the Xac-Aw strain by conjugation was not successful. In an attempt to locate possible avirulence genes, a genetic library of Xac-A^w DNA was made using the pLAFR vector and transformed into *Escherichia coli*. This library was transferred to strain 91-118 of *X. perforans*, which is pathogenic to tomato, but causes a null reaction in grapefruit leaves. The transconjugants were screened for HR in grapefruit leaves. The inoculated leaves were observed for development of an HR in the infiltrated area. Three clones eliciting HR in grapefruit leaves were conjugated into an Asiatic (A) citrus canker strain, which is pathogenic in grapefruit and key lime. Only one clone (799) when expressed in the A strain caused an HR in grapefruit. Furthermore, it did not cause an HR in key lime. A 2.1 kbp subclone contained the active gene, which was sequenced. PCR primers to the gene amplified DNA of all A^w strains, but none of the A strains. When the gene was inactivated in the parent strain by marker exchange the mutant strain caused typical disease symptoms in grapefruit leaves. The HR reaction was restored by complementation of the mutant with the 799 subclone. Therefore, the *avr* gene appears to limit the host range of A^w strains

C-29 GENETIC DIVERSITY AND WORLDWIDE PROLIFERATION OF CITRUS BACTERIAL CANKER PATHOGENS IDENTIFIED IN HISTORIC SPECIMENS

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Citrus bacterial canker (CBC) caused by *Xanthomonas axonopodis pv. citri* (Xac) may have originated in Southeast Asia, based on symptoms present on early herbarium specimens. The disease was first introduced into the United States in 1911 and has spread to most citrus producing areas in the world. This serious disease now threatens the world citrus industry, especially the $10 billion Florida industry. Regulatory emergencies triggered by the appearance of new strains make it increasingly important to understand the origins, evolution and phylogenetics of this pathogen. No information was available on the genetic diversity of the pathogen during the earlier expansion of the range of the pathogen. Herbaria are important resources for the study of the origins and dispersal of plant pathogens. USDA-ARS at Beltsville, Maryland maintains 741 herbarium specimens of citrus with symptoms typical of CBC, originally from 36 citrus producing countries in the world. Since DNA in herbarium specimens is often degraded and citrus plant materials contain compounds that inhibit PCR amplification, we have developed an efficient and reproducible protocol for DNA extraction. We then selected a total of 90 specimens representing 20 citrus and related species from the herbarium collection. Based on the full genomic sequence of reference strain Xac306 of the pathogen, we designed a comprehensive set of primers targeted at transposable element insertion sites located near strain-specific genes. Our method, which we call Insertion Event Scanning (IES), should become a powerful tool for the study of bacterial population genetics. To demonstrate the power of the method we have obtained invaluable data which document the substantial genetic diversity of CBC pathogens deposited as herbarium specimens worldwide during the 20th century. The primary, secondary and tertiary proliferation centers of CBC pathogens have been identified in Japan, the Philippines and India, respectively. Most of the CBC emergencies in citrus producing areas in the new world have been traced back to their origins from the proliferation centers in the old world. A map of the likely routes taken by the bacterium during the course of its global expansion has been developed. IES represents a novel and general approach to the study of bacterial diversity and will be immediately applicable to all bacterial taxa as full genome sequence data becomes available. The method will also be useful in the analysis of the origins and management of contemporary outbreaks of any previously characterized pathogen.

C-30 Genetic Studies of Resistance to Citrus Canker

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Susceptibility and resistance to citrus canker, caused by *Xanthomonas axonopodis* p. *citri*, has been evaluated over time by observations of field infections and by artificial inoculations. These investigations have revealed that most *Citrus* species and cultivars are susceptible to ACC, but kumquats (*Fortunella* spp.), Ichang papeda (*C. ichangensis*), and Calamondin (*C. mitis*) have been shown to be highly resistant; most mandarins (*C. reticulata*) such as Ponkan, Satsuma, Tankan, and Sunki are considered to be tolerant. Our group has studied the resistance associated with both kumquat and *C. ichangensis*. We have cloned genes of the receptor kinase class (RLK) of disease resistance genes from citrus BAC libraries and used these gene sequences to design primers to probe both kumquat and Ichang papeda genomes for homologous genes; RLK genes in plants include *Xa-21*, a gene conferring broad spectrum *Xanthomonas* resistance in rice. Crosses of susceptible Palestine sweet lime (*C. limettoides*) and resistant Ichang papeda have been made and evaluated for canker resistance. A continuous phenotypic segregation was observed suggesting quantitative and possibly polygenic control of resistance. A total of **88 AFLPs and the RLK homologues** associated with the canker resistance have been mapped. Another line of research has revealed an incompatible reaction between *Fortunella margarita* and the canker pathogen, confirmed by bacterial growth curves. Forward and reverse subtractive cDNA libraries were constructed using Nagami kumquat mRNA to identify genes that are differentially expressed during the interaction, and most of these cDNA clones were sequenced. Homologues to transcription factors, receptors, and resistance genes known to be involved in plant-pathogen interaction were identified. cDNA microarrays containing these genes were analyzed for expression profiles during the interaction, to elucidate pathogenesis-related response mechanisms and to assign roles to previously uncharacterized genes. Identification of natural resistance resources and isolation of disease resistance genes offer a potential to overcome the devastating threat of this disease.

C-31 MOVING CITRUS CANKER RESISTANCE FROM KUMQUAT INTO TRIPLOID ACID-FRUIT AND MANDARIN HYBRIDS VIA INTERPLOID CROSSES

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Interploid hybridization is an efficient method for generating improved seedless triploid citrus hybrids for the fresh market. The production of numerous high quality tetraploid breeding parents of various parentage via somatic hybridization has facilitated application of this approach for the improvement of acid-fruit hybrids (lemons/limes), mandarin hybrids, and grapefruit-like hybrids. One objective of our interploid breeding program is to move citrus canker resistance from kumquat into improved seedless acid-fruit and mandarin hybrids. Interploid crosses involving the purportedly canker resistant 'Lakeland' limequat were conducted to generate acid-fruit triploids. Resistance of citrus genotypes to *Xanthomonas axonopodis* pv. *citri,* the cause of Asiatic citrus canker (ACC), was evaluated by injection infiltration of 10^3 and 10^4 cfu/ml through stomates on the abaxial surface of immature leaves. Citrus genotypes for screening included two autotetraploids and nine triploid hybrids of 'Lakeland' limequat (*Citrus aurantifolia* (Cristm.) Swing. x *Fortunella japonica* (Thumb.) Swing.) and their progenitors ['Lakeland' limequat, the autotetraploids 'Femminello' lemon (*Citrus limon* (L.) Burm. f.) and 'Giant Key' lime (*C. aurantifolia* (Cristm.) Swing., and the somatic hybrids 'Key' (also known as 'Mexican') lime + 'Valencia' orange and 'Hamlin' orange + 'Femminello' lemon]. 'Meiwa' kumquat (*Fortunella crassifolia* Swing.) and 'Pineapple' sweet orange (*C. sinensis* (L.) Osbeck) were used as known resistant and susceptible standards, respectively. Lesion number per inoculation site and bacterial population per lesion were recorded 15-19 days after inoculation. The assay was performed four times during a spring-summer-fall period under greenhouse conditions. Canker lesions were consistently produced by stomatal inoculation with 10^4 but not 10^3 cfu/ml. Susceptible and resistant genotypes were separated based on lesion number per inoculation site and bacterial population per lesion. Spearman's rank correlation analysis for lesion numbers on 15 genotypes common to all four assays showed significant correlations among the genotype rankings. Genotype rankings were also significantly correlated between the two bacterial population assays. Lesion number per inoculation site is sufficient for assessment of resistance of citrus genotypes to ACC without the necessity of conducting bacterial population assays. These results indicate that 'Lakeland' limequat is a promising seed parent for breeding acid citrus fruit that is resistant to ACC. Additional somatic hybrid allotetraploid breeding parents containing 'Lakeland' limequat have been produced, including 'Lakeland' limequat + 'Key' lime, 'Lakeland limequat + Jaboticaba tangor, and 'Lakeland' limequat + 'Lapithotiki' lemon. Interploid crosses to move kumquat-derived canker resistance into triploid mandarin hybrids have also been conducted, and a cross of the acidless 'Succari' sweet orange with the somatic hybrid 'Meiwa' kumquat + 'Changsha' mandarin yielded 4 viable triploid hybrids following embryo rescue. These hybrids are being propagated for the canker challenge assay.

C-32 Genetics of citrus canker host / pathogen interactions.

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Every citrus canker strain, regardless of origin or "form" of canker caused by the strain, carries at least two DNA fragments that hybridize with *pthA*, a member of the *avrBs3/pthA* gene family. Five functional *pthA* homologues, *pthA*, *pthB, pthC, pthAW and pthA** were cloned and sequenced from all known citrus canker groups: A, B, C, A^w and A* respectively. All fully complemented a *pthA*::Tn*5* knockout mutation, and without evidence of avirulence function. Unlike nonfunctional homologues tested that were not required for citrus canker disease, all *pthA* homologs required for canker had 17.5, nearly perfect, 34 amino acid direct repeats. The amino acid sequence of the 17^{th} repeat may be determinative of ability to cause citrus canker disease.

By itself and without benefit of any other bacterial gene, *pthA* is capable of causing citrus canker disease symptoms when expressed in citrus cells, making PthA and all family members from *X. citri* with 17.5 direct repeats unusually effective plant effector proteins. These effectors are directly targeted to the plant nucleus, affecting transcription and effecting citrus hyperplasia and cell death. Approximately 500 citrus cDNA clones were tentatively identified by subtractive suppression hybridization and differential display experiments as responsive to PthB. A total of 46 of these citrus genes were found to be differentially regulated by PthB. Not surprisingly, the citrus canker effectors appear to up-regulate specific genes involved in cell wall structural remodeling.

C-33 Rapid screening of anti-bacterial gene products in citrus trees using a CTV-based transient expression vector.

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We developed a transient expression vector based on an infectious cDNA clone of *Citrus tristeza virus* (CTV) to produce foreign gene products in citrus trees. The vector can express high levels throughout citrus plants of an open reading frame (ORF) of a foreign gene inserted into the genome of CTV and controlled by an extra subgenomic mRNA promoter. The foreign gene is expressed systemically in both shoots and roots of trees. After the vector has been amplified to infect an tree initially, it can be graft inoculated to other citrus trees of any size or variety. This is an effective technology to rapidly screen genes for potential use to control diseases caused by the canker or huanglongbing bacterium. In a first series of experiments, we chose ten different anti-bacterial peptides systemically expressed in citrus trees to be tested for activity against these organisms. The CTV-based vector produces the foreign proteins in phloem-associated cells, which coincides with the location of the huanglongbing bacterium. However, in order to target the peptides to the location of the canker bacterium, we modified each of the peptides with one of two different leader peptides to transport the peptides across membranes and cell walls.

In the initial experiments, six peptides without leader peptides significantly reduced production of canker lesions in citrus leaves. Reduction of lesions ranged from 50 to 80 percent of the untreated inoculated control.

C-34 Production Of Transgenic Plants Expressing The NPR1 Defense Gene To Develop Resistance To Citrus Canker

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Systemic acquired resistance (SAR) is an important defense mechanism in plants. SAR is associated with the induction of pathogenesis-related (PR) genes and confers broad-spectrum resistance against a variety of pathogens (virus, bacteria and fungi). One of the central genes involved in SAR is the *Arabidopsis NPR1* that functions as a signal modulator [1-4]. Upon induction (by pathogen infection and/or salicylic acid, SA), *NPR1* expression is elevated and the NPR1 protein is activated, in turn inducing expression of a battery of downstream PR genes. NPR1 interacts in the nucleus with members of the basic leucine zipper (bZIP) family of transcription factors (TGA factors) to induce the activity of PR genes [5-7]. Because the sequence of the *Arabidopsis NPR1* gene was available and it has been demonstrated that its overexpression in *Arabidopsis* and other heterologous systems (rice and tomato) induces broad spectrum disease resistance [8,9], we cloned this gene from *Arabidopsis* genomic DNA and used it in plant transformations. Subsequently, we also cloned an *NPR1* homolog from sweet orange (*Citrus sinensis*). Initially most of our transformations were made with Carrizo citrange because of seed availability and because this rootstock is easy to regenerate and is fast growing. We have 26 Carrizo and one grapefruit confirmed transgenic plants that are rooted and are being propagated. Analysis of the expression of the transgenic plants using quantitative RT-PCR indicated that the *Arabidopsis NPR1* gene is expressed and the host *PR1* is expressed at higher levels compared to nontransgenic plants, at least in some of the lines. The plants are morphologically normal and appear healthy. *PR1* is considered a marker for SAR and the SA pathway. This is a promising result since we expected the transgenic lines to have an enhanced defense response compared to the nontransgenic plants. The most important characteristic of our approach is that using defense genes of plant origin may lead to broad-spectrum protection against a variety of pathogens. In addition, since they are of plant origin, they should be of less concern to consumers. We are currently in the process of evaluating these plants for their resistance to canker.

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C-35 Enhanced Resistance to Citrus Canker in Transgenic Sweet Orange Plants Expressing the Antibacterial Peptide Sarcotoxin IA

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Different strategies have been proposed in order to improve resistance to bacterial diseases in plants by genetic engineering, including producing antibacterial proteins of non-plant origin, inhibiting bacterial pathogenicity or virulence factors, enhancing natural plant defenses and artificially inducing programmed cell death at the site of infection. The constitutive expression of antibacterial peptides from insects is the strategy that has shown the most promising results for control of phytopathogenic bacteria.

Sarcotoxins are cecropin-like antibacterial peptides isolated from larvae of the common flesh fly, *Sarcophaga peregrina*. They belong to a type of antibacterial peptide in which two amphiphilic α-helices interact with bacterial cell membrane, causing loss of membrane electrochemical potential. Among this group, sarcotoxin IA, a peptide consisted of 39 amino acids, is the most characterized one. *In vitro* experiments showed that synthetic sarcotoxin IA inhibited the growth of different phytopathogenic bacteria at low concentration, including *X. axonopodis* pv. *citri*.

Transgenic Pera sweet orange (*Citrus sinensis* Osbeck) plants constitutively expressing the sarcotoxin IA peptide were obtained by *Agrobacterium*-mediated gene transfer using thin sections of mature explants. The sarcotoxin IA gene fused to the signal peptide of PR1a from *Nicotiana tabacum* for secretion to the intercellular space was driven by the 35S CaMV constitutive promoter. Thirteen independent transgenic lines were obtained. Preliminary evaluation of citrus canker resistance in leaves of transgenic and non-transgenic sweet orange plants was performed through infiltration of a 10^5 cfu/ml bacterial suspension. A significant increase in resistance to citrus canker bacterial infection was observed in transgenic plants expressing high levels of sarcotoxin IA peptide.

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C36 - Protocols for Orchard and Packinghouse Sanitation to Qualify for Fresh Fruit Shipments

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In order to preserve fresh fruit markets in the face of expanding canker quarantine zones in production areas, current regulations require that fruit be harvested from recently inspected canker-free blocks followed by separate handling procedures backed up by treatment with surface sanitation agents in the packinghouse. This presentation will describe the currently prescribed and accepted procedures necessary to qualify fruit for shipment to another citrus production area, whether domestic or foreign. Questions to be addressed are:

1. Why is it important to sanitize fruit surfaces? How much inoculum is there? Does it pose a risk?
2. How are canker-blemished fruit kept out of the fresh fruit product stream? What are the consequences of failure here?
    a. Visual inspection of block prior to and during harvest
    b. Visual inspection on packinghouse line
3. How much reduction in inoculum on canker blemish-free fruits can one achieve with present methods? How much reduction is necessary to make shipments safe for other citrus production areas?
4. How can the efficacy of fresh fruit surface sanitation be measured?
    a. Empirical testing on artificially inoculated fruit
    b. Bacteriophage testing
    c. ELISA on population-amplified wash water
    d. Flow cytometry

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Packing House Compliance Agreement
http://www.doacs.state.fl.us/onestop/forms/08358.pdf

Harvester / Handler Compliance Agreement
http://www.doacs.state.fl.us/onestop/forms/08359.pdf

The Second International Citrus Canker and Huanglongbing Research Workshop

Contributed Papers on Huanglongbing H1-H16

H-1 Huanglongbing: Biology and Overview

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Huanglongbing ("yellow dragon disease", HLB) was first observed in China in the late 19th century. During the 1920s, South African farmers noted a citrus malady which they named "yellow branch" in one area, and "greening" in another. The infectious nature of HLB and greening were not initially suspected. However, we now know that these two diseases are forms of one disease, known officially as HLB. They share general symptomatology and transmission methods, but differ in the symptom severity and temperature sensitivity. The range of symptoms associated with HLB includes a range of chlorotic foliar effects, while fruit are small, lopsided and have poor color development and a bitter taste; the Asian form can eventually lead to tree death. Both forms can result in catastrophic crop losses.

Although Koch's postulates have not been fulfilled because of the inability, so far, to culture the causal organism, it is widely accepted that HLB is caused by a phloem-limited bacterium of the *Candidatus* genus Liberibacter. The Asian species, *Cd*. L. asiaticus, is naturally transmitted by the Asian citrus psyllid, *Diaphorina citri*. Both insect and bacterium can tolerate temperatures above 30°C, and thrive in hot, low lying areas. They occur together throughout the Asian citrus growing countries from Pakistan in the west to southern Japan in the east. They have also been reported in the Arabian peninsular and the Indian Ocean islands of Reunion and Mauritius. The African form is associated with *Cd*. L. africanus, and is transmitted naturally by another psyllid species, *Trioza erytreae* – both are intolerant of temperatures above 30°C, and therefore are found at higher elevations. African HLB is found in southern, central, eastern and western Africa, as well as in the Arabian peninsular and the Indian Ocean islands.

D. citri has been present in South America since the 1940s, and has recently spread north into the Caribbean, Central America, Florida and Texas. In 2004 HLB symptoms were reported in citrus trees in Brazil. Although a few trees were found to be infected with *Cd*. L. asiaticus, the vast majority were infected with a new species, *Cd*. L americanus. In 2005 HLB symptoms were found in Florida, and *Cd*. L asiaticus has been identified.

Much research on HLB remains to be done. With better detection techniques to improve the understanding of the interrelationships between the pathogen, the plant hosts and the vectors, it should be possible to develop more effective control strategies.

H-2 Huanglongbing in São Paulo State, Brazil : challenges and achievements, March 2004-September2005.

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Symptoms of huanglongbing (HLB) were recognized for the first time in the Araraquara region of São Paulo State (SPS), Brazil, in March 2004 (1). They included yellow shoots, leaves with blotchy mottle, and lopsided fruits with colour inversion, aborted seeds, and brownish vascular bundles. In a few farms, the percentage of affected trees was high, suggesting that the disease has probably been present in SPS since several years, but was incorrectly diagnosed. Today, HLB is present in 79 municipalities from SPS and 1 from Minas Gerais, but in most farms the number of affected trees is below 0.05%. The major causal agent of HLB in SPS is a new liberibacter species, *Candidatus* Liberibacter americanus, but the Asian liberibacter, *Candidatus* Liberibacter asiaticus, is also present. From March 2004 to May 2005, 596 trees were found infected with liberibacters, of which 539 (90.4%) carried *Ca*. L. americanus, 38 (6.4%) carried *Ca*. L. asiaticus, and 19 (3.2%) were infected with both liberibacters. Characterization of *Ca*. L. americanus as a new species was based on the phylogenetic analysis of the gene for 16S ribosomal RNA, as well as on the sequence comparison of the liberibacter 16S/23S ribosomal intergenic region (2). PCR primers for the detection of the two SPS liberibacters have been published (3). The two liberibacters can now be detected concomitantly in the same PCR tube. Midribs from leaves with symptoms of blotchy mottle represent the most reliable plant material for PCR detection of the liberibacters. The Asian citrus psyllid, *Diaphorina citri*, vector of *Ca*. L. asiaticus, and reported as early as 1942 from SPS, could be shown to be infected with *Ca*. L. americanus, and is very probably vector of the American liberibacter too (3). The rutaceous ornemental shrub and tree, *Murraya paniculata*, frequently seen in SPS and the preferred host plant of *D. citri*, was shown to be infected with *Ca*. L. americanus as well as *Ca*. L. asiaticus (Sylvio Lopes, Fundecitrus, personal communication), and might influence the epidemiology of HLB. Efforts to combat HLB in SPS are based on chemical control of *D. citri*, and compulsory, law-reinforced, continuous removal of symptomatic trees. The difficulty comes from the fact that trees, which have been infected, but show no symptoms yet, cannot be easily identified in the field. Frequent surveys are necessary to pick up these trees as soon as they start showing symptoms. In China, common experience shows that without these control measures (chemical control of the psyllids and tree uprooting), an HLB-infected orchard is destroyed within 5 to 8 years. Examples of such situations will be presented.

1) Teixeira et al. 2005. First report of a huanglongbing-like disease of citrus in São Paulo State, Brazil, and association of a new Liberibacter species, "*Candidatus* Liberibacter americanus", with the disease. Plant Disease. 89: 107.
2) Teixeira et al. 2005. , "*Candidatus* Liberibacter americanus", associated with citrus huanglongbing (greening disease) in São Paulo State, Brazil. International Journal of Systematic and Evolutionary Microbiology 55 : 1857-1862.
3) Teixeira et al. 2005. Citrus huanglongbing in São Paulo State, Bra zil: PCR detection of the *Candidatus* Liberibacter species associated with the disease. Molecular and Cellular Probes 19: 173-179.

H-3 The Discovery of Huanglongbing in Florida

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Diaphorina citri Kuwayama was found in Florida for the first time in June 1998 in Delray Beach, Palm Beach County. The infestation had spread to central Broward County to the south and southern Martin County to the north along the Florida East coast prior to its discovery. We estimate that *Diaphorina citri* had been in Florida for 6 months to 1 year before it was found. *Diaphorina citri* colonized Southeast Florida in the ensuing 6 months, after which it spread to the remainder of the state on potted plants (primarily *Murraya puniculata*) in retail trade.

Intensive surveys of southern Palm Beach County several years after the discovery of *Diaphorina citri* indicated that there was no detectable huanglongbing. Similarly, an intensive survey in 2000 in Homestead turned up no positive plant. DPI sentinel tree surveys by fruit fly detection personnel (mainly in Pinellas County, Hillsborough County and Orange County) turned up no huanglongbing suspect.

In 2004, the Florida Cooperative Agricultural Pest Survey (CAPS) team began intensive sentinel surveys for huanglongbing in Orlando and Tampa Bay, targeting neighborhoods with high populations of people from parts of the world where huanglongbing is endemic. These surveys turned up no plant positive for huanglongbing.

In 2005, the CAPS team initiated targeted surveys of Asian farms for exotic pests of all kinds. On 24 August 2005, a pummelo tree with suspect huanglongbing symptoms was found in Florida City. Another citrus plant (unknown cultivar) with huanglongbing symptoms was found in Palmetto Bay about 18 miles north of the plant in Florida City. Both plants were diagnosed as positive for huanglongbing through PCR analysis at the Division of Plant Industry in Gainesville and confirmed by the USDA.

Subsequent surveys by the combined USDA/DPI Incident Command determined that the disease had spread throughout residential areas in Miami-Dade, Broward, Palm Beach and Martin counties prior to the initial two detections. Additionally, there were infected plants in several commercial groves to the west of the residential areas.

H-4 Current status of citrus HLB and efforts toward its eradication in São Paulo State, Brazil

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The presence of citrus HLB in Brazil was confirmed in July 2004. The disease was demonstrated to be caused by the phloem limited bacterium *Candidatus* Liberibacter asiaticus and by the new species *Ca.* L. americanus. With the description of the new Liberibacter species, new PCR primers were designed for pathogen detection and HLB diagnosis (1) which have been used in research and on the ongoing HLB eradication program. Originally, plants showing HLB symptoms were detected in March 2004 in the vicinity of Araraquara county located at the central region of the State of São Paulo (SP). After confirmation of the presence of the disease in Brazil, Fundecitrus conducted surveys to identify and quantify the affected areas. In October 2005, HLB had been detected in 79 municipalities of the State of SP and in one municipality of the State of Minas Gerais (MG). Ninety six percent of all symptomatic plants found so far were distributed in 10 municipalities in the central region of SP. In collaboration with public institutions, Fundecitrus has been working on an intense research program on HLB diagnosis, epidemiology, alternate hosts and pathogen transmission, and on the biology and control of the insect vector. Parallel to these efforts, Fundecitrus initiated a communication campaign in TVs, radios and newspapers with the objective to call growers attention on the importance of the disease and symptom recognition, and on the necessity of planting healthy young trees, performing vector control, and prompt elimination of the symptomatic plants to reduce inoculum sources. The growers have also been advised to not prune affected plants due the inefficacy of this practice in controlling the disease (2). Currently in SP, all young trees are produced in nurseries protected against insect vectors, which represent a great improvement in the control of citrus diseases, including HLB. Up to now more than 4000 growers and technicians participated of Fundecitrus oral presentations on HLB and more than 1500 inspectors were trained in the recognition of HLB symptoms throughout SP State. In March 2005, the Brazilian Ministry of Agriculture published a Normative Instruction making mandatory the elimination of all citrus plants showing HLB symptoms as well as *Murraya paniculata* which may also host the HLB pathogen (3). In May 2005 the Secretary of Agriculture Protection of the State of SP, in collaboration with Fundecitrus and Centro APTA Citros "Sylvio Moreira", initiated the HLB eradication program. This program involves 735 inspectors and technicians to inspect all citrus trees of all SP and MG citrus farms, collect samples, and run PCR analyses for correct diagnosis. As a result of all these efforts, the majority of the symptomatic trees (ca.300 thousand) identified so far has been deliberately eliminated by the growers. The objective is to inspect all proprieties of the 80 affected municipalities until February 2006, and to eliminate all symptomatic plants detected.

1) Teixeira et al. 2005. Citrus huanglongbing in São Paulo State, Brazil: PCR detection of the *Candidatus* Liberibacter species associated with the disease. Molecular and Cellular Probes 19: 173-179.
2) Lopes et al. 2005. Ineficácia da poda no controle do huanglongbing dos citros. Fitopatologia Brasileira 30:64.
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H-5 Huanglongbing: its possible origins, collaborative research in Southeast Asia, and developing incursion management plans for Australia

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The Australian Centre for International Agricultural Research and Horticulture Australia Limited are funding research in Indonesia and Việt Nam on huanglongbing (citrus greening) and its vector, the Asiatic citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Psyllidae). The key objectives involve development of incursion management plans for the Australian citrus industry, surveys of psyllids and their natural enemies, and studies to determine: (a) distributions of known and potential alternative hosts of the disease and the psyllid; (b)
(c) impacts of ambient temperatures at different latitudes, longitudes and altitudes on the incidence of the psyllid, and the spread and severity of huanglongbing in different species and cultivars of *Citrus* and alternative hosts; (d) impacts of plant nutrition on disease development; (e) impacts of adult psyllid feeding behaviour and host plant phenology on transmission of the disease to sweet orange (*Citrus* × *aurantium* L. Sweet Orange Group), mandarin (*C. reticulata* Blanco) and alternative hosts within the Rutaceae; (f) impacts of sprays of mineral and plant oils on psyllid populations, and spread of the disease, through direct effects on mortality of adults and nymphs and the indirect impacts of deposits on feeding and oviposition; and (g) interactions between scion genotype, rootstock and huanglongbing.

The project commenced in 2002. Progress is being made, despite the impact of external factors such as terrorism and more fundamental scientific issues that were not fully appreciated when the project commenced, namely the origins of *Citrus*, huanglongbing and *D. citri*, and the systematics of *Citrus* and its allies. It is widely assumed, largely because of hopelessly out of date and incorrect texts, that *Citrus* originated in a region encompassing modern India and Southeast Asia. It is also widely assumed that huanglongbing originated in China. We believe both assumptions are false. Current evidence indicates that of some 30 true species of *Citrus* approximately 40% are Asian and 60% Australasian. We hypothesise that *Citrus* evolved in eastern Gondwana and that subsequent speciation occurred in Asia and Australasia. Current evidence also suggests (a) that the Diaphorineae and the bacteria that cause huanglongbing have Gondwanan origins and (b) that spread of *D. citri* and *Candidatus* Liberibacter asiaticus eastward from the Indian subcontinent is related mostly to trade by sea over the past 400 to 1000 years[but mostly within the past 2 to 3 centuries.

There is an urgent need to resolve confusion related to the plethora of species names used for apomictic hybrid clones of *Citrus*. There is a profound misunderstanding of generic limits and nomenclatural chaos engendered by utilising taxonomic systems based on up to 157 species names for the genus. Furthermore, there is an urgent need to resolve issues related to the taxonomic status of *Citrus* relatives and their susceptibility to huanglongbing, particularly *Murraya* (*sensu stricto*) within the Aurantieae, and *Bergera* and *Clausena* within the Clauseneae.

H-6 - Huanglongbing Epidemiology: Tracking the Dragon Through Time and Space

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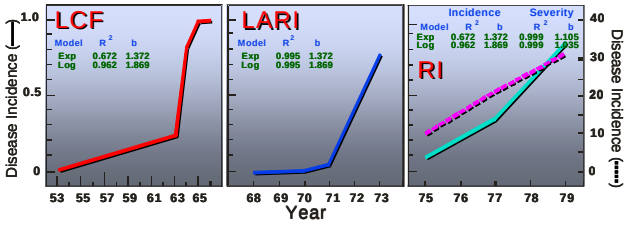
The epidemiology of Huanglongbing (HLB) caused by *Candidatus Liberibacter asiaticus* (*CLa*) has been observed and written about, but few quantitative epidemiological studies have been conducted. This is due to the perennial nature of the disease requiring a dedication to data collection over multiple years and the inability, until recently, of detection by PCR, requiring a reliance for disease monitoring on visual assessment. Monitoring the occurrence of symptoms, can be problematic. The lag in time between transmission by psyllid vectors or propagation and the onset of symptoms can be quite variable and quantifying the severity of disease symptoms in individual trees may not be a true indication of pathogen concentration. Additionally, due to the temporal variation in symptom expression, trees infected at the same time may express symptoms over one or more years. This variable lag period, compromises the accuracy of spatial and temporal studies. Symptoms observed are the expression of infections that have occurred over multiple years in the past. Thus we are visually assessing a 'fuzzy history' of infection that has occurred over a prior time period equivalent to the lag period. Nevertheless, epidemiological information can be used to predict the economic and physical life of a given planting and provide a means to investigate the efficacy of potential control interventions.

The spatial and temporal dynamics of HLB were investigated in plots in Reunion Island (RI) and southern China (LCF, LARI) to estimate the rates of disease increase and expected longevity of infected sweet orange and mandarin groves infected with *CLa.*

Analysis of disease progression. HLB epidemics are multiyear in duration and rarely progress to an asymptote before removal of the planting occurs. Therefore both the exponential and the logistic models adequately described disease progress over time. In the LCF plot an asymptote was reached after 13 yrs. But for groves that became unproductive and were removed, disease incidence only reached 0.76 after 6 yr (LARI) and 0.84 after 9 yr (RI), but the logistic model predicted asymptotic disease levels (0.99) after 7 and 13 years for these plots respectively.



Spatial analysis of HLB data. Spatial pattern analysis was undertaken to better understand the relationships among infected trees near to and at distance from each other and thereby gain some understanding of vector spread of HLB, and was accomplished by examining the HLB data at various spatial levels (hierarchies). Combined interpretations of spatial analyses indicate two mechanisms of vector spread of HLB, within local areas and over longer distances. Within local areas, aggregations of infected trees occur that at times can be quite large (up to 1672 trees). In this case vectors apparently spread the disease to either adjacent or nearby trees. Spatial autocorrelation also identifies a prevalence of reflected clusters or areas of aggregation that are discontinuous with the main cluster. These are interpreted as indicative of the presence of secondary foci that are quite prevalent and are at a distance of about 25-50 m from the main cluster of disease and each other. Such a pattern of widely spaced foci perhaps indicates a spatial mechanism associated with longer distance vector movement. Longer regional scale vector transmission has not been investigated.

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H-7 Spatial Distribution of Huanglongbing Symptomatic Trees in Citrus Groves in São Paulo, Brazil

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Huanglongbing (HLB) was reported in 2004 in São Paulo, Brazil and *Candidatus* Liberibacter asiaticus and *Ca.* L. americanus, were found associated with the disease with prevalence of the second species. The Asian psyllid vector of *Ca.* L. asiaticus, *Diaphorina citri* Kuwayama, reached Brazil 60 years ago, is well established in São Paulo orchards, and is probably the main candidate of HLB-agent vector in Brazil. However, due to its recent report in Brazilian orchards, the transmission of *Ca.* Liberibacter ssp. by *D. citri* in Brazil is not yet confirmed and no data is available with regard to HLB spatial distribution in Brazil. Assessments of diseased trees by visual symptoms were made in 36 groves from 8 farms in the central citrus region of São Paulo State. 155 HLB spatial maps (varying from 0.14 to 25.99% of disease incidence) were analysed, considering quadrat sizes of 2x2, 4x4, 6x6 and 8x8 trees, by ordinary runs analysis, binomial index of dispersion and binary form of Taylor's power law. Aggregation among HLB-symptomatic trees was detected by ordinary runs analysis, with clustering existed in both within- and across-rows directions. However the percentage of aggregation within- and across-rows were low. The binomial index of dispersion for various quadrat sizes suggested aggregation of HLB-symptomatic trees for about 40% of the plots. The relationship between log(observed variance) and log(binomial variance) was highly significant for all four quadrat sizes. Estimated parameters of the binary form of Taylor's power law provided an overall measure of aggregation of HLB-symptomatic trees for all quadrat sizes tested. All power law estimates of *b* and *A,* were statistically different from 1, which indicated a general and significant pattern of aggregation of symptomatic plants for all quadrat sizes tested. The degree of aggregation was also positively related to disease incidence. Data from 20 plots ranging in disease incidence were also analyzed by spatial autocorrelation to examine the association among groups of infected trees using the 2x2 quadrat size. In 14 of 20 cases, clusters of HLB-infected trees were found to be associated with secondary clusters whose centers were at distances ranging from 4.2 to 22.1 tree spaces distant, indicating psyllid vector movement resulting in transmission both to nearby trees causing clusters and to trees at considerable distance initiating new foci of infection.

H-8 Diagnostics of huanglongbing : detection of the causal liberibacters, *Candidatus* Liberibacter asiaticus, *Ca*. L. africanus, and *Ca*. L. americanus, in plants and insects by electron microscopy, DNA hybridization, and PCR.

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Huanglongbing (HLB) is caused by endogenous, sieve tube restricted, Gram negative bacteria (1), which were discovered by electron microscopy (EM) in 1970, and have, so far, resisted *in vitro* cultivation. Their molecular characterization as liberibacters could be achieved only when DNA-based techniques became available in the early 1990s. Hence, for many years, EM detection of the HLB bacteria was the only reliable laboratory method to diagnose HLB in citrus from many different countries (2). It was based on two basic properties of the HLB bacteria: they were exclusively located in the sieve tubes, and, contrary to mycoplasmas, they possessed a clearly visible cell wall of the Gram negative type. In citrus, up to now, no agents other than the HLB bacteria have been found to fit these properties.

In 1981, the HLB bacteria could be transmitted from citrus to periwinkle plants (*Catharanthus roseus*) by dodder (*Cuscuta campestris*). Total DNA from infected periwinkle plants served to clone genomic DNA fragments of the Asian HLB bacterium (strain Poona, India). One of the cloned HLB-DNA fragments, named In-2.6, was part of the bacterial β-operon (*rpl* genes for ribosomal proteins and genes for β + β' subunits of RNA polymerase). In-2.6 was used to obtain, from an African HLB bacterium (strain Nelspruit, South Africa), a similar β-operon fragment, named As-1.7. Used as DNA probes, In-2.6 and As-1.7 were able to specifically detect the Asian HLB bacterium and the African HLB bacterium, respectively (3, 4). Dot-blot hybridization was thus the first molecular technique used to detect the HLB bacteria in citrus. These probes have also been used to detect the HLB bacteria in individual psyllids on "crush-blots". Finally, In-2.6 and As-1.7 were used to define a pair of PCR primers, *rpl* A2 and *rpl* J5, which yield a 667bp amplicon with the African HLB bacterium, and a 701bp amplicon with the Asian agent (5).

Molecular characterization of the Asian and African HLB bacteria was reported in 1994 and 1997 (6, 7). The gene coding for 16S ribosomal RNA (16S rDNA) was obtained by PCR amplification with universal primers fD1/rP1, and sequenced. The HLB 16S rDNA sequences were used for the phylogenetic and taxonomical characterizations. The HLB bacteria were found to represent a new bacterial genus within the α-*Proteobacteria* (Gram negative bacteria): the genus *Candidatus* Liberibacter (*Ca*. L.), with two species: *Ca*. L. asiaticus for the Asian HLB agent, and *Ca*. L. africanus for the African agent. Forward primers OIn1 + 0Af1, and reverse primer OIn2c were designed from the HLB 16S rDNA sequences. With both liberibacter species, the same 1160bp amplicons are obtained. However, the amplicon from the Asian liberibacter has one *Xba* 1 restriction site and yields two fragments upon digestion, while the African species has two such sites and yields three fragments, making liberibacter identification straight forward (8).

With either one of the two primer pairs, *rpl* A2 / *rpl* J5 or [OIn1 + 0Af1] / OI2c, the Asian and the African liberibacters can be detected most reliably in leaves showing HLB blotchy mottle, but not in symptomless leaves. These PCR methods make it possible to confirm HLB in suspicious trees, but they should not be used for indexing purposes.

Unexpectedly, in April 2004, when HLB was suspected in São Paulo State, the above primers yielded negative PCR reactions with many leaf samples, even though these leaves showed strong blotchy mottle. This result led to the discovery of a new

liberibacter species: *Candidatus* Liberibacter americanus. Primers f-GB1 and r-GB3 were developed from the 16S rDNA sequence of the new species. These primers are specific for the American HLB, as they do not detect the the Asian and the African liberibacters.

In São Paulo State, from August 2004 to September 2005, *Ca*. L. americanus was detected in 1411 trees (92,5%), *Ca*. L. asiaticus in 82 trees (5.2%), and both liberibacters in 32 trees (2.1%).

A duplex PCR method, with primers GB1/GB3 and *rpl*A2/*rpl*J5 for the simultaneous detection of *Ca*. L. americanus and *Ca*. L. asiaticus in the same PCR tube, has been developed and used routinely.

Nested PCR with fD1/rP1 as the first primer pair, and GB1/GB3 or [GB1/GB3+ *rpl*A2/*rpl* J5] as the second pair(s) has been compared to conventional PCR, especially in an attempt to detect *Ca.* L. americanus in symptomless leaves. The results will be discussed.

As of September 2005, HLB affects 79 municipalities in São Paulo State, and has reached 1 municipality in Minas Gerais State.

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H-9 COMPARISON OF METHODS FOR THE DETECTION OF *CANDIDATUS* LIBERIBACTER ASIATICUS IN PLANT SAMPLES

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The Exotic Pathogens of Citrus Collection (EPCC) at Beltsville maintains an inventory of several hundred potted citrus trees infected with exotic, graft transmissible pathogens, including *Ca.* Liberibacter spp. Plants from the EPCC were selected and used to compare published PCR-based detection methods for *Ca.* Liberibacter spp. with Loop Mediated Isothermal Amplification (LAMP) and a new Taqman based assay for quantitative, real time PCR. The 76 trees selected for the study included trees entered into the collection with *Ca*. Liberibacter spp. as the pathogen of record, as well as trees entered with other pathogens of record. The tree samples also included sub-propagations of the original accessions. DNA was extracted using the Qiagen Plant DNeasy kit from ~200 mg of the proximal portion of midribs pooled from three mature leaves from each tree. Symptomatic tissue was chosen when present. PCR amplification with PCR primers A2/J5 (Hocquellet et al., 1999) and OI1/OI2C; GB1/GB3 (Teixeira et al., 2005) was carried out first, followed by LAMP (Okuda et al., 2005) and RT-PCR (Li et al., submitted). Primer pairs A2/J5 and OI1/OI2C each detected *Ca.* Liberibacter asiaticus in the same 15 trees (20%). LAMP assays also detected the pathogen in the same 15 trees. The Taqman assay also detected the pathogens these fifteen trees, as well as one additional tree. *Ca*. Liberibacter asiaticus could be detected in a 10^{-5} dilution of extracts, using the Taqman assay. Primer pair GB1/GB3 did not detect *Ca*. Liberibacter americanus in any of the trees, as expected. *Ca*. Liberibacter africanus was also not detected in any sample, although trees thought to be infected with this species were tested. We also noted two instances where the original accession tested negative for *Ca*. Liberibacter asiaticus, but sub-propagants were positive. It is likely that these trees were cured of *Ca.* Liberibacter spp. by high greenhouse temperatures. We further noted several instances where different sub-propagants from a source tree gave different reactions, suggesting that transmission by grafting is not 100 % efficient. In our hands, the LAMP assay was prone to contamination, yielding obvious false positives. The fact that the four assay systems gave congruent results suggests that each assay system is reliable.

H-10 Development of Multiplex Real-Time PCR for Detection and Identification of *Candidatus* Liberibacter Species Associated with Citrus Huanglongbing

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Citrus huanglongbing (HLB, ex greening) is one of the most serious diseases of citrus in the world. There are three forms of the disease caused by three species of *Candidatus* Liberibacter, *Ca*. L. asiaticus (Las), *Ca.* L. africanus (Laf), and *Ca.* L. americanus (Lam). The diseases can be transmitted by psyllids and by grafting. The psyllid vector *Diaphorina citri* can transmit both Las and Lam. Establishment of this vector into Florida, reports of Lam and Las in Brazil in 2004, and recent confirmation of HLB in Florida is of great concern to the citrus industry. Research on HLB has been hampered by the unculturable nature of the causal bacterium in artificial media. It has been difficult to detect and identify these pathogens because of their low concentration and uneven distribution in host plants and vector psyllids. In this study, we developed a quantitative TaqMan PCR using 16S rDNA-based primers and probes specific to each species of HLB pathogens and an additional COX-based primer and probe set as positive internal control to assess the quality and quantity of DNA extracts from environmental samples. The assays do not cross-react with other pathogens or endophytes commonly resident in citrus plants, and are very sensitive with a detection limit of HLB pathogen DNA obtained from 20 ng of midrib tissue from symptomatic leaves. Results of assays with DNA extracted from plants infected by various species or strains of these pathogens in greenhouse- and field-grown material demonstrated high repeatability and reproducibility. There was no effect of plant inhibitors on assay results. Based on the TaqMan PCR quantification, the bacterial population of the pathogens was estimated to be 2.5×10^7 and 2.0×10^6 cells per gram of fresh midribs of symptomatic leaves from sweet oranges infected by Las and Lam, respectively. The ratio of the pathogen DNA to host plant DNA was estimated to be 1:1,000 in DNA extracts obtained by the standard CTAB method. This rapid, sensitive and specific TaqMan PCR for detection, identification and quantification of HLB pathogens has been successfully used with other assays in the first confirmation of HLB in Florida, and should prove useful for research, regulatory activities, and management of the disease.

H-11 Detection and Identification of Citrus Huanglongbing (Greening) in Florida, USA

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Citrus Huanglongbing (HLB), one of the most devastating diseases of citrus, was first found in South Florida in August, 2005. The causal agent of the Florida HLB was identified as *Candidatus* Liberibacter asiaticus based on sequence analysis of its 16S rDNA, 16S/23S intergenic region, and β-operon. These sequences were PCR-amplified from infected plant tissues using primers CG03F and CG05R (Duan *et. al*., unpublished), OI1 and OI2c (Jagoueix *et al*. 1994, 1996), OI2 and 23S1 (Jagoueix *et al*. 1997), and A2 and J5 (Hocquellet et al, 1999). Liberibacter asiaticus has been detected by PCR both in its insect vector, *Diaphorina citri* and the infected citrus plants, including pummelo (*Citrus maxima*), grapefruit (*C.* x *paradise*), sour orange (*C. aurantinum*), sweet orange (*C. sinensis*), lime (*C. aurantifolia*), lemon (*C. limon*), kumquat (*Fortunella japonica*), and calamondin (X *Citrofortunella microcarpa*). However, we did not have the evidence to demonstrate that the HLB bacteria multiply in *Murraya paniculata*, the most important host of the psyllids. In addition, negative or weak PCR results from many symptomatic samples using the above-mentioned primers indicated possible variability of the HLB pathogen in Florida.

H-12 Overview of the Asian Citrus Psyllid

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The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae), was first found in Florida during June 1998 and is now widespread throughout much of the state. This invasive psyllid vectors the bacterium responsible for huanglongbing (HLB) (greening disease), a serious citrus disease. HLB was found in Florida late August 2005. The presence of *D. citri* in Florida sets the stage for further spread of the disease in Florida and possibly into other North American areas. *D. citri* has a restricted host range that includes citrus, orange jasmine and related species of Rutaceae. Continuous flushes produced by jasmine could play a role in psyllid populations in nearby citrus. Orange jasmine is not considered an HLB host, but this deserves confirmation. Adult *D. citri* are small (2.7 to 3.3mm long) with mottled brown wings and are active, jumping/flying insects that can readily move short distances. Adult flight can occur all day long but may be pronounced during warm, windless, sunny afternoons between 4 and 6 pm; flight is inhibited by windy weather. Speculations are that flying adults could be transported by prevailing winds over a 0.5 to 1 km distance depending on duration of sustained flight. Another psyllid, *Trioza eryteae* in Africa, may fly 1.5 km with the help of prevailing winds in absence of host plants. Adults on leaves hold their heads to the leaf surface with their bodies at a 30-45° angle to the leaf surface. Eggs are deposited on terminal flush growth and are elongated (0.31mm long) and oval in shape, initially light yellow but at maturity bright orange with two distinct red eye spots. Numerous eggs can be found on flush, each anchored to plant tissue on one end generally in an upright position. There are five nymphal stages. At 25°C, eggs hatch in 4 days, nymphs develop into adults in 13 days, and new adults reach reproductive maturity within 15 to 17 days for a mean population generation time of 32 to 34 days. Adults live an average of 40 to 48 days, during which females continuously lay eggs if flush is present. Maximum adult longevity ranges from 117 days at 15°C to 51 days at 30°C. A sex ratio of about 1:1 has been reported. Adult females lay an average of 858 eggs on grapefruit. Population fluctuations of *D. citri* are closely correlated with flush growth because oviposition and development of nymphs take place exclusively on flush. Visual surveys by persons trained to recognize *D. citri* may be the fastest way to detect an infestation. There is no attractant available for survey purposes. Sticky traps have some value in monitoring adults; their general efficacy depends on color and placement. Information on sampling to estimate infestation densities is available for citrus and jasmine. *D. citri* in Florida is subjected to natural control by an array of predators and one introduced parasitoid species, *Tamarixia radiata.* However, notable infestations of the pest continue to occur. Carefully timed insecticides may provide some control of HLB. The HLB pathogen can be acquired by an adult within a 30 min feeding period and transmitted during a 5-7 hr feeding period. Adults can transmit the pathogen many days after acquiring it. Late instar nymphs can also transmit HLB, and they retain the pathogen after becoming adults. Ovarial transmission of the pathogen has been speculated but remains to be confirmed. When the psyllid was first found in Florida, surveys indicated it was already present in four counties, too widespread for eradication efforts. Successful eradication might not have been possible even if the psyllid had only been present in one small area.

H-13 Studies on *Campylomma chinensis* Schuh, a Potential Biocontrol Predator of *Diaphorina citri* Kuwayama

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Campylomma chinensis Schuh (Hemiptera: Miridae) is a natural enemy of many insect pests on citrus (*Citrus reticulata* Blanco), *Canarium album* Raeusch, and *Murraya exotica* L , lonyan (*Euphoria longana* lam) and lychee (*Litchi chinensis* Sonn). It can prey many kinds of insect eggs of Lepidoptera and Homoptera, such as *Diaphorina citri* Kuwayama, *Cormegenapsylla sinica* Yang et Li. *Pseudophacopteron canarium* Yang et Li, and *Conopomarpha sinensis* Bradley. The predator was first found in Fujian Province, China, in 1984. In 2003, we began our research on mass rearing of *C. chinensis* Schuh, especially on their diet. The effects of 17 kinds of diets were tested. Two of them were the best that allow *C. chinensis* Schuh complete their nymph stage. The rates of survival were above 81.11%. The life span of the third generation had little difference from that of the first generation. Releasing *C. chinensis* Schuh to control *Diaphorina citri* Kuwayama on citrus orchard and *Conopomorpha sinesis* Bradley in *longan orchard* showed that *C. chinensis* Schuh could prey 400 eggs *of Diaphorina citri* Kuwayama and 3000 eggs on citrus tree all over their life. They could significantly decrease the number of survival eggs of *Diaphorina citri* Kuwayama after six days released.

H14 - Management of Asian citrus psyllid on citrus with ADMIRE PRO, PROVADO, and TEMIK

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A multi-tiered approach for the management of citrus greening involves the use of clean budwood, planting of healthy nursery stock, eliminating insect vectors, and prompt elimination of infected trees to reduce inoculum sources for further spread of the pathogen. Field evaluations with ADMIRE PRO, PROVADO, and TEMIK from Florida, Texas, Brazil, and Thailand have shown excellent initial and residual control of Asian citrus psyllid, a vector of citrus greening. These products serve as powerful tools for protecting citrus budwood sources and nursery stock, as well as eliminating the vector on both immature and mature field plantings.

H-15 Strategies for sequencing the genome of *Liberibacter*, an uncultured Select Agent.

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In early 2004, USDA-APHIS funded a project to obtain 8X coverage of the entire genome of *Ralstonia solanacearum* Race 3 Biovar 2 (R3B2), a Select Agent. The genome and initial machine annotation was performed commercially in a matter of months, and the data was made immediately available to a select group of international researchers for manual annotation, data mining and improved diagnostics via a secure web site (http://www.vision.biotech.ufl.edu). By late 2004 the first draft of the publication (Gabriel et al., 2005, Molec.Plant-Microbe Interactions, in press) was written. Availability of the R3B2 genome allowed immediate, prepublication improvements in PCR diagnostics and also insight into R3B2 genomic variation, including identification of genes involved in host range, pathogenic severity and ecological fitness. The general strategy for sequencing and annotation involved: 1) independent validation of strain identity and pathogenicity; 2) independent lab curation at Select Agent containment facilities; 3) commercial sequencing and contig assembly; 4) commercial machine annotation, and 5) distributed manual annotation and data curation. A total of 93,580,574 base pairs (bps) were sequenced. A cosmid library was also constructed in a broad host range shuttle vector to help close gaps in the assembled contigs, to make particular genomic regions available to the research community, and for functional genomics experiments. A total of 768 cosmid clones were curated, end-sequenced and mapped to the contigs.

The complete genomic DNA sequence of a *Liberibacter* strain is a current research priority for at least five reasons. First, all current PCR tests for citrus greening are confirmatory only, and also require DNA extractions because of poor sensitivity. Sequencing an entire *Liberibacter* genome may help increase sensitivity by discovery of multicopy PCR target loci within the genome. This may obviate the need for DNA extraction, increase the throughput, and make possible detection of *Liberibacter* prior to the appearance of symptoms. Second, all current PCR tests are based upon just two (2) gene loci. Having an entire genome available would assist in the design and validation of many additional potential primer sets, avoiding both false negative and false positive PCR results. Third, additional loci would assist in the detection of new strains or even species of *Liberibacter*. The extent of strain variation within this putative, "Candidatus", genus is unknown at present. Fourth, the complete genomic sequence should reveal potential pathogenicity mechanisms and thereby provide a basis for more effective chemical or genetic controls. Finally, once the first genome is completed, rapid genomic comparisons with additional, perhaps unusual strains become possible. For sequencing of a *Liberibacter* genome, a similar general strategy as used for the R3B2 genome sequencing project could be followed, but with much additional care required at the validation, curation and DNA extraction stages due to inability to isolate, purify and culture the microbe. In the absence of ability to grow purified strains of *Liberibacter* in culture, extracts from citrus likely need to be enriched for phloem sap and prokaryotic genomic DNAs. The sequence assembly process combined with methods specifically designed to amplify circular genomes should facilitate the project. Potential complications include mixed *Liberibacter* infections in the same plant and contaminating endophytic bacteria in greening-infected citrus.

H-16 Screening of anti-bacterial peptides in citrus trees for activity against *Candidatus* Liberibacter asiaticus

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Citrus huanglongbing disease (HLB) has been recently reported from both Brazil and Florida. Because of the uniquely destructive nature of the disease, and the present lack of options to control the disease, new approaches are needed. The bacterium that causes HLB, *Candidatus* Liberibacter asiaticus, is found in the phloem vessels of infected plants. Although the pathogen is a true bacterium, it has thus far not been possible to culture the bacterium in vitro, and so conventional antimicrobial screening methods are not applicable. Strain T36 of *Citrus tristeza virus* (CTV) was modified so that it could encode and express a series of 8 small, antibacterial peptides in citrus plants. Because CTV occupies the phloem vessels of infected citrus, as does *Ca*. Liberibacter asiaticus, this approach allows us to screen the antibacterial against *Ca*. Liberibacter asiaticus to determine which, if any, of the peptides might warrant further research in terms of transgenic citrus. Budwood containing the modified CTV constructs was received from Florida and propagated on *Citrus macrophylla*, Citron, and sweet orange 'Madame Vinous' in a secure greenhouse in Beltsville, MD. Infection by the CTV constructs was confirmed by ELISA. These plants were then challenge-inoculated by grafting with *Ca.* Liberibacter asiaticus containing budwood in May, June and July of 2005. Plants were maintained and evaluated for symptoms of HLB. Samples were then collected and the amount of *Ca.* Liberibacter asiaticus DNA present in each plant was quantified by quantitative real-time PCR. Our results are preliminary at this time, as at least one year of growth and evaluation may be needed to see definitive results.

The Second International Citrus Canker and Huanglongbing Research Workshop

Contributed Posters on Citrus canker and Huanglongbing P1-P24

P-1 Citrus Canker exposure expansion in residential areas of Palm Beach County after Year 2004 hurricane season.

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Gregory J. Monaghan. Division of Plant Industry, SEFL CCEP Program Director.
Richard Miranda. Division of Plant Industry, SEFL CCEP Public Information Director.
Mark J. Fagan. Division of Plant Industry, SEFL CCEP Public Information Spokesman**.**

Rationale The fight against Citrus Canker disease (*Xantomonas axonopodis* pv. *citri* (Asian strain) threatening Florida's Citrus Industry, an ongoing effort was severely impacted by weather conditions during 2004 hurricane season.

Objectives The objective of this study is to compare residential exposure areas before and after 2004 hurricane season.

Methods Independent program database (PICS) queries, comparing data as of July 1, 2004 and as of February 28, 2005 (12 weeks after the end of 2004 hurricane season).

Results A dramatic increase in exposure arcs expansion was seen in the results obtained for February 28, 2005 as result of four hurricanes hitting south and central Florida in year 2004.

Conclusions These data indicate the importance of supporting survey and control efforts to remove all exposed trees within 1900 feet of any positive finding at faster pace prior to major weather events.

Citations: Division of Plant Industry, SEFL CCEP statistics, 2005.

P-2 Success of Program control results based on increased public education efforts (waiver-runners).

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Richard Miranda. Division of Plant Industry, SEFL CCEP Public Information Director.
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Rationale The fight against Citrus Canker disease (*Xantomonas axonopodis* pv. *citri* (Asian strain) threatening Florida's Citrus Industry, has achieved excellent results due to increased public information efforts during years 2004 and 2005.

Objectives The objective of this study was to demonstrate the effectiveness of public information efforts resulting in the ability to control the vast majority of residential trees encompassed by exposure arcs without the need to obtain agricultural warrants.

Methods Independent program database (PICS) queries, comparing warrants execution data as of July 31, 2004 and as of July 31, 2005.

Results A proven positive response from the public sensitized by Florida's economic threaten.

Conclusions Obtaining owners' consent to remove positive and exposed trees in residential areas of SEFL, has positively impacted the program's effectiveness by enabling cutting crews to sweep through entire areas in just one visit while limiting the impact on the Court system.

Citations: Division of Plant Industry, SEFL CCEP statistics, 2005.

P-3 Citrus Canker exposure expansion as consequence of three years of Court constraints.

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Rationale The fight against Citrus Canker disease (*Xantomonas axonopodis* pv. *citri* (Asian strain) threatening Florida's Citrus Industry, an ongoing effort, was severely hampered for three years by Court constraints imposed by a Broward County Judge.

Objectives The objective of this study is to demonstrate the negative impact of legal constraints placed on the eradication program by comparing residential exposure areas prior July 2000 to residential data collected once full operations resumed in 2004.

Methods Independent program database (PICS) queries, comparing data as of July 31, 2000 and as of July 31, 2004.

Results A dramatic increase in exposure arcs expansion in Palm Beach County was observed in year 2004 after three years of restricted operations by Court order.

Conclusions Legal constraints placed on the eradication program facilitated the spread of the disease. The establishment of a buffer area in the northern tiers of Palm Beach County as a measure to protect Florida's Citrus Industry may curtail additional spread, but will not mitigate the impact of three years of court constraints.

Citations: Division of Plant Industry, SEFL CCEP statistics, 2005.

P-4 Functional Genomics and Diagnostic of *Xanthomonas axonopodis* pv. *citri* Towards Pathogenicity-Related Genes

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During the complete genome sequencing of *X. axonopodis* pv. citri (Xac), the causal agent of the citrus canker disease, many sequences related to pathogenesis/virulence and with similarity to various bacterial plant pathogens genes were found at the nucleotide and amino acid level. However, only a few of such sequences were experimentally evaluated to reveal their biological function. To this end, we have developed a multidisciplinary approach that utilizes the pathogen itself or its interaction with the host plant to establish "cause and effect" relationships between the biological condition and measurable molecular/genetic alterations. Basically, in our laboratory and with collaborations, the functional analysis of Xac has been based on mutagenesis and gene disruption, macroarrays, and proteomics analyses. Screening of a high-density transposon insertional mutant library of Xac 306 against the citrus host plant (*Citrus sinensis*) has allowed the identification of several single-copy insertions in protein-coding sequences (CDSs) and intergenic regions that have resulted in altered virulence. Moreover, other genes potentially involved with pathogenicity have been submitted to site-directed mutagenesis and their role during infection has been analyzed, including genes involved in secretion of effector proteins. To uncover how sets of genes and their products work together in disease, we also developed a macroarray analysis that aimed to identify among all 279 genes annotated as pathogenicity-related their expression under conditions that, supposedly, mimic the plant intercellular space. The results indicate a system that could be used as an *in vitro* system to identify candidate genes involved in pathogenesis of Xac. Also, a 2-D protein gel electrophoresis approach has been used for the identification of modifications in the protein pattern due to the gene mutations. Concerning to citrus canker diagnosis, a new PCR-based test was developed with primers that anneal to a Xac-specific region inside the *rpf* gene cluster. A 581-bp fragment was amplified from DNA of strains belonging to Xac from different regions around the world, including unusual American and Asian strains. This specific 581-bp PCR product was not obtained with DNA extracted from strains of the closely related *X. a. aurantifolli* and *X. a. citrumelo*, neither with extracts prepared from 28 xanthomonads of other species and epiphytic bacteria isolated from citrus. Amplification was obtained from cells grown *in vitro*, from extracts of both fresh and dried citrus canker lesions, and from washes of inoculated but asymptomatic leaf surfaces.

P-5 The distribution of citrus canker in Emerald, Australia and bacterial survival in citrus trash

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Citrus canker was first detected on a commercial citrus property in Emerald, Queensland in June 2004; 'infested premise 1' (IP1). Disease on a second property was reported by the owners in October 2004 (IP2) and disease surveillance detected a third infested premise (IP3) in May 2005.

The disease was estimated to have established on IP1 possibly as early as 2002, based on evaluation of the growth rings of infected stems. At IP2 and IP3, symptoms were not present on mature stems, so the time of introduction was estimated from the association of symptoms with successive growth flushes, at no earlier than summer 2003/2004.
To determine secondary disease spread on IP1, trees along transects through the three worst-affected blocks were inspected and rated. On IP2 and IP3, studies involved identifying the point of primary infection and rating all trees surrounding that point. Disease severity was recorded for all trees inspected. The results of each study are summarised in Table 1.

Table 1: Summary of citrus canker data from the three infested properties.

Property	Block	Date of disease detection	Maximum disease severity (%)[1]	Disease incidence (%)[2]	Oldest infected growth flush	Proposed mechanism of secondary spread
IP1	1D	July 2004	>25	94	Unknown	Unknown
	2D	July 2004	6-25	100	Prior to 2004	Unknown
	5D	July 2004	6-25	52	Unknown	Unknown
IP2	Pivot 7	Oct 2004	>25	3.9	Not prior to 2004	Pivot irrigator
	Pivot 8	Nov 2004	>25	1.6	Not prior to 2004	Pivot irrigator
	41	Feb 2005	2	0.4	Spring 2004	Unknown
	1	March 2005	25-50	1.0	Autumn 2004	Wind-driven rain or spray applicator
	36	April 2005	3-10	3.2	Spring 2004	Farming equipment
IP3	Rootstock	May 2005	>50	3.9	Autumn 2004	Wind-driven rain or spray applicator

[1] The disease severity is the proportion of the total foliage of a tree that has symptoms

[2] The disease incidence is the proportion of infected trees to the total number inspected

Citrus canker lesions were excised from fallen leaf and fruit material and evaluated for viable *X. axonopodis* pv. *citri*. The pathogen was cultured from 8% of leaf (n = 25) and 9% (n = 66) of fruit lesions obtained from fallen material whereas 82% (n = 22) of leaf and 69% (n = 13) of fruit lesions collected from live hosts had viable bacteria. Lesions and bacterial cultures were indexed by PCR and the presence of *X. axonopodis* pv. *citri* confirmed for all.

P-6 Evaluating potential alternative hosts of citrus canker

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Citrus canker was detected on three properties within a citrus production area in Queensland Australia during 2004/5. As part of the disease eradication strategy a series of experiments were conducted to identify alternative hosts within the Pest Quarantine Area (PQA). This information will also be valuable in the event of further disease incursions.

A range of native and ornamental rutaceous species plus other reported hosts were evaluated in *in vitro* bioassays. Selected leaves were surface sterilised and wound inoculated with fresh suspensions of pathotype A strains of *Xanthomonas axonopodis* pv. *citri* (syn. *X. smithii* subspecies *citri*). Symptoms were initially recorded descriptively then converted to a numerical ranking of zero to four.

All six Australian native Citrus species and four rutaceous species were confirmed as hosts of the bacterium. Six of these ten species are new host records and these are indicated by asterisks in Table 1. Plants from these species that are detected within the PQA will be removed. A further eight native rutaceous species were evaluated and found to be non-hosts. The results of completed experiments are summarised in Table 1 and a further 21 species are currently undergoing evaluation.

Table 1: A summary of plant species evaluated in bioassays and their status as an alternative host of *X. axonopodis* pv. *citri.*

Plant species	Status
Acronychia acidula *	Host
Citrus australis	
Citrus australasica	
Citrus garrawayi *	
Citrus inodora	
Citrus gracilis *	
Citrus glauca	
Lunasia amara *	
Micromelum minutum *	
Murraya ovatifoliolata *	
Clausena brevistyla	Non-host
Clausena smyrelliana	
Dinosperma sp.	
Flindersia collina	
Flindersia dissosperma	
Geijera parviflora	
Glycosmis trifoliata	
Halfordia kendack	
Luvunga monophylla	
Murraya paniculata ‘exotica’	

P-7 The truth and facts pertaining to the handling of the Citrus Canker outbreak in Emerald, Australia from an Emerald citrus grower's perspective.

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The following abstract aims to highlight what, from an Emerald citrus grower's perspective, were the major short comings in the handling of the Citrus Canker outbreak in Emerald, Australia. In doing so, it will create an awareness of seldom mentioned facts and effects to the citrus growers and their families.

Citrus Canker was officially detected in Emerald in June 2004. To date there are still living citrus trees located in the Pest Quarantine Area awaiting destruction. As the power to order the eradication of all host material lay with the Queensland Government, responsibility for the delay in achieving a swift eradication of all host material lies directly with that Government and relevant Government Departments. It is considered that the Governments actions and the lack thereof were a result of their lack of experience in dealing with this particular exotic incursion.

Upon the initial detection at IP1, a surveillance method to give a 95% probability of detection was implemented by authorities on the remaining orchards in the area. This method was refuted by growers as unreliable and was ultimately shown to be flawed with the disease later being found by a citrus grower, not surveillance teams. The grower that initially found the second outbreak had, prior to the discovery, self funded their own study tour of other countries to research Canker, as the DPI&F denied access to photographic literature relating to the initial outbreak on IP1 or to see samples thereof. Information from data collected from the growers' properties has not been passed to growers in a timely manner and at times has been unobtainable. Queensland Government staff has at times also refused to communicate with growers on the basis of instructions received from their employer not to do so.

Upon the detection of Canker at IP2, the owners of that farm, brokered an eradication plan with all remaining commercial growers on the 5/10/04 and presented it to the DPI&F on the 7/10/04 to preemptively eradicate all commercial citrus trees in the PQA. The cost at that time to carry out complete host eradication would have been $16 million (AUD). The large yet isolated community had offered complete eradication for the good of the entire Australian citrus industry and to ensure a quick return to citrus production. The proposal was dismissed in favor of the Governments own interpreted version of the Florida protocol that was devised to suit their own agenda. The protocol was falsely promoted as being based on 'science'. With further detections within the PQA, the preemptive common sense approach,(which became known as the 'Pressler Plan') where all host trees are removed in the PQA irrespective of disease symptoms, was eventually adopted over the so called 'scientific' approach. This Government eradication program is currently being implemented at a cost of $11.5 million (AUD).To date the total budgeted allowance is $19.4 million(AUD), not including a further $5 million (AUD) already spent by DPI&F outside of the program.

In summary, the cost of eradication has risen from a possible $16 million (AUD) to $35.9 million (AUD), with a loss of 15 months before growers can re-establish orchards. Only growers with trees that were removed after 3/ 6 /05 are entitled to a reimbursement of $80 / tree (plus a nominal re-establishment payment) with Government Tax yet to be subtracted. Growers will not receive any reimbursement or assistance to re-establish trees destroyed prior to this date.

Government action and treatment of the growers has created an environment of fear. Fear of how you will be treated if you have, or you are suspected of having Citrus Canker. Growers and residents alike are subject to sizable fines for not conforming to hastily implemented regulations. It is extremely unfair that a Government can remove the earning capacity of citrus growing families by quarantine measures without firm assurances for re-entry to previously established markets. Unfortunately, it has been heard that growers from other regions of Australia would not report detections of Citrus Canker to authorities given the poor treatment experienced by Emerald growers at the hands of the authorities.

For future handling of exotic incursions many lessons can be learned from the Australian experience. Effective communication and transparent dissemination of information with growers is essential. The outcome of complete eradication of the incursion must be paramount. Budgetary constraints must be amended to meet the highest level of eradication, not as has happened in Australia where the level of control of the disease appears to be commensurate with the budgetary allowance.

P-8 A Concise History of Citrus and Citrus Canker in Texas

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In the 1890s, the major citrus belt in Texas was along the Gulf Coast from Houston to Beaumont. Though two tree-killing freezes in 1894 and 1895 destroyed nearly every tree, the industry was revived, only to be hit by another major freeze in 1899. It revived once again, but a 1916-17 freeze finally crippled the citrus industry in this area and citrus production shifted to the Lower Rio Grande Valley (LRGV). Some citrus is still grown in the Gulf Coast area in small orchards and as dooryard trees for local consumption. The first commercial grove in the LRGV was 7-acres of oranges, limes, and lemons near Brownsville, planted in 1891 by a French immigrant. The first grapefruit grove on sour orange rootstock was planted in 1908. There was rapid growth of the industry during and after the 1920s.

The canker bacterium, *Xanthomonas axonopodis* pv. *citri* was accidentally introduced into the United States through trifoliate orange seedlings and satsuma trees shipped from Japan in the early 1900s to Texas, Mississippi, Alabama, and Florida. In Texas, canker was first found in Alvin, near Houston. Subsequently, canker was found on trifoliate orange trees shipped from Texas to Florida. Nurseries were reportedly established in Texas and Florida with canker-infected citrus trees. Infected trees were subsequently distributed to other states. After a serious eradication campaign with the burning of infected trees, canker was eradicated in Florida and in Texas in 1933 and 1943, respectively. In the Gulf Coast States, from Florida to Texas, nearly 20 million trees in nurseries and groves had been destroyed by 1934 because they were infected or exposed to citrus canker. As late as 1949, surveys and inspections were still being conducted in the Gulf region on scattered, residential and wild trees, and abandoned groves. In 1999, surveys were conducted in 10 counties in both the Gulf Coast and the LRGV areas for canker and citrus leaf miner (CLM), *Phyllonistis citrella,* damage. No canker lesion was detected in any of the more than 1,328 *in situ* samples; 122 samples tested negative in the laboratory. Active CLM infestations and severe foliar feeding injury were apparent at most survey locations. In 2000, another survey for citrus canker was conducted in Cameron County in the LRGV. A total of 822 samples were collected and assayed, 790 had leafminer damage. Only one sample, a grapefruit leaf, showed suspicious symptoms with brown circular raised lesions on the upper surface and surrounded by a clear yellow chlorotic halo. The characteristic water-soaked margin and sunken center of citrus canker lesions were not seen. In the laboratory, bacterial streaming was observed from thin sections cut at the junction of the lesion and healthy tissue. However, bacterial tests using a selective medium did not confirm presence of canker. In 2003, a survey of 81 locations in the Gulf Coast region with several thousand wild trifoliate seedlings showed no evidence of the presence of citrus canker.

P-9 Microarray Expression Profiling of Nagami Kumquat during its Incompatible interaction in Response to Canker

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Citrus canker disease, caused by a bacterial pathogen *Xanthomonas axonopodis* pv *citri*, affects a variety of citrus species and citrus relatives in many areas of the world. Kumquats have shown an apparent resistance to canker that has not been previously unraveled. In this study, an incompatible interaction of *Fortunella margarita* and *Xanthomonas axonopodis was* confirmed using bacterial growth curves. Further, forward and reverse subtractive cDNA libraries were constructed using Nagami kumquat mRNA to identify genes that are differentially expressed during the interaction with the canker pathogen. Some cDNA clones were selected for sequencing, and the sequences were analyzed using NCBI BLAST. Homologues to transcription factors, and receptor and resistance genes known to be involved plant-pathogen interactions were identified. cDNA microarrays containing 2304 of these genes were analyzed for expression profiles during the interaction with the canker pathogen. The results demonstrate how the use of microarray-based expression profiling methods can help elucidate plant pathogenesis-related response mechanisms and assign roles for previously uncharacterized genes. This can be considered to be a case study in citrus that has used these high throughput technologies to understand the defense mechanisms in *Fortunella* and citrus at the molecular level.

P10 Quantitative trait linkage (QTL) mapping for resistance to citrus canker within Citrus

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The majority of agronomically important phenotypes including disease resistance are quantitative, influenced by multiple genes as well environmental factors. A bacterial pathogen, *Xanthomonas axonopodis* pv. *citri* (*Xac*), is an immediate threat to the entire Florida citrus industry. The individuals of a mapping family, designated the 9100 family from the cross between susceptible Palestine sweet lime (*Citrus limettoides*) and resistant Ichang papeda (*C. ichangensis*), revealed a normal distribution from the most resistant to most susceptible individuals to the inoculation of citrus canker bacteria. Based on this phenotyping result, a study was conducted to locate quantitative trait loci (QTL) conferring resistance against citrus canker using markers developed by amplified fragment length polymorphism (AFLP). We applied an AFLP approach, combined with bulked segregant analysis (BSA), to an F1 population and their parents using one hundred and six pairs of AFLP primer combinations. A total of 94, from the determined 1,105 polymorphic bands (present in the resistant parent and absent in the susceptible parent), were found tightly associated with the phenotyped resistant individuals. A molecular local linkage map consisting of 94 AFLPs was developed by Joinmap v.2.0 and detected QTLs were located on the linkage map by MapQTL. QTL detection is a first step in this research giving information about the genetic control of complex adaptive traits. The screening of these citrus canker resistant related marker is currently underway using a backcross family from the cross between susceptible Clementine mandarine (*C. reticulata*) and the most resistant individual (3-17-12) of the 9100 family.

P-11 A comparison of image analysis and visual assessment of citrus canker symptoms

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Citrus canker, a disease of several citrus species, is caused by the bacterial pathogen *Xanthomonas axonopodis* pv *citri* (*Xac*). The disease is of concern in several wet tropical and subtropical citrus growing regions as infection results in yield loss and severely blemished fruit unsuitable for the fresh market (4). Ways of managing the disease are being sought, and accurate, precise and reliable disease assessment is needed for monitoring epidemics. Visual assessment (VA) is the only reliable means estimating disease. The objective of this study was to compare image analysis (IA) to VA for assessing symptoms of citrus canker (1,3).

Figure 1. Example of the relationship between incidence (lesion no.) and severity (%leaf area) of infection for 214 grapefruit leaves. Data is for VA2.

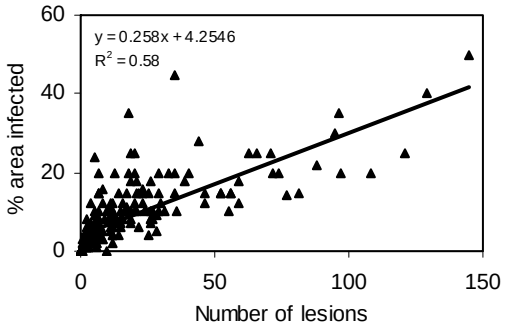
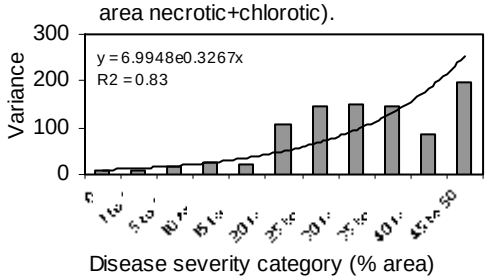


Figure 2. Variance in visual assessments of disease severity (% area necrotic+chlorotic).



Digital images of 214 citrus leaves with a range of incidence and severity of citrus canker were assessed by three plant pathologists (VA1-3) and by image analysis (IA), and the results analyzed using general linear modeling (GLM) and regression analysis. All four assessments showed only a moderate relationship between lesion number and % area infected (R^2=0.55-0.68, Figure 1), suggesting that appropriate assessments (lesion counts or % area infected) should be chosen to suit the objectives of the experiment. GLM analysis showed no significant differences between IA and VA for lesion number (mean 16.3-18.7 lesions per leaf), although there were significant differences in severity using % area necrotic (3.3-5.4%), but not % area chlorotic+necrotic (8.9-11.0% mean area). This may be due to VA being less capable at differentiating necrotic areas but being better at assessing the whole infected area. Using IA as a standard, variance of the VA assessment increased as lesion number increased and as severity increased to 50% area (Figure 2), agreeing with previous observations on perception of disease severity (2): the more disease there is (at least up to 50%), the less accurate the assessment.

A sample of 53 leaf images was converted to black/white (b/w) and IA/VA performed. Both image sets were assessed for incidence and severity with comparable accuracy by VA1-3. However, variance tended to be greater for color images suggesting that the greater degree of subjectivity in identifying and delimiting disease where color is involved (although difference between image type may also be due in part to the b/w images standardized by IA).

IA appears to provide a reliable way to assess canker infected leaves for disease, but symptom characters (heterogeneity, coalescence of lesions) combined with assessors loss of concentration, can lead to discrepancy in results.

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P-12 Construction and Deployment of a Mobile Containment Greenhouse/Laboratory

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Functional field laboratory and greenhouse space is often at a premium in emergency programs. To help provide mobile field facilities for the Citrus Canker program, a collaborative project between USDA-ARS and USDA-PPQ was initiated. The concept of a mobile containment greenhouse/laboratory (MCGL) was originally developed by two ARS scientist, T .Gottwald and S. Garnsey, 9 years ago. A prototype unit was built and has functioned as a containment greenhouse/laboratory for citrus canker studies in the Miami area for a number of years. The usefulness of this concept became apparent and improvements to the original prototype design were proposed for a second generation prototype. The new design, a self-contained, semi-mobile, computer-controlled unit, was constructed by USDA, APHIS, PPQ. The 3 inch double walls of the greenhouse are constructed of custom extruded aluminum framing sheathed with double layer Lexan panels. Environmental parameters (temperature, lighting, CO_2, and watering) are continually monitored and controlled by a computer data acquisition and feedback control system. Basic laboratory equipment includes: a refrigeration/freezer, biosafety cabinet, incubator, autoclave and a HEPA air filtration unit. An emergency generator functions automatically as a power back-up and can also provide full electrical power in the absence of local electricity. Other features include a holding tank for effluent, metal diamond plate floors, central channel drain, irrigation/fertigation system for the greenhouse plants and external field plots.

The bid for construction of this unit was awarded in September of 2003 and actual construction of the greenhouse laboratory commenced later that year. The unit, completed as to contract specifications, was delivered to the APHIS, PPQ Aircraft Equipment Operations group (AEO) in Edinburg, TX in April of 2004. AEO provided the skills and labor to accomplish the final outfitting of the MCGL. This second generation prototype Mobile Containment Greenhouse/Laboratory is currently being used for citrus canker work, but more importantly, can be replicated and rapidly deployed as needed by APHIS as outbreaks of new high consequence quarantine pests occur in the U.S. *Photographs: upper-MCGL, middle- laboratory, lower- greenhouse*

P-13 The change in quantity of bacteria of *Xanthomonas axonopodis* pv *citri* dispersed down wind from canker-infected grapefruit trees during a wind/rain event

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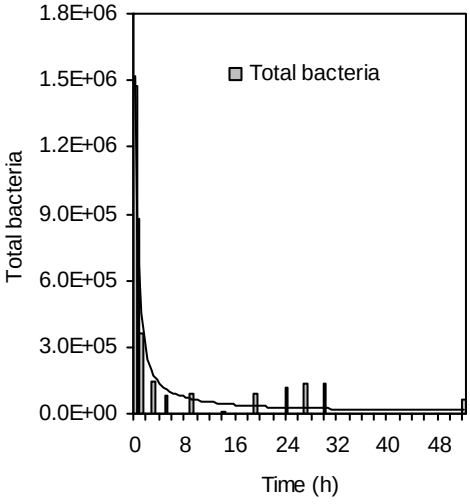
The yield and marketability of citrus is limited in several tropical wet parts of the world by citrus canker (caused by *Xanthomonas axonopodis* pv. citri, *Xac*). The disease can cause severe epidemics and there are few options for control, although eradication has been favored (1). A thorough knowledge of the epidemiology of *Xac* can contribute to improving the eradication program and eventual management of citrus canker. There is limited information available on the dispersal of bacteria in the field during rain showers (which are often associated with strong winds, especially in tropical storms and hurricanes). The aim of this work was to investigate the dynamics of dispersal of the bacteria from infected plants during wind-rain events.

Wind was simulated using yard-blowers (11-27 m/sec, depending on experiment), and spray (4.5 l/min) was generated using sprayers directed horizontally into the canopy of canker-infected plants (2). Panel and funnel samplers collected splash down wind and under the plants, respectively. The volume collected was measured and bacteria colony counts were made on nutrient agar and related to wind duration using regression analysis.

Figure 1. Relationship between the quantity of bacteria of *Xac* dispersed and time in an experiment monitoring dispersal over 52 h.



Bacteria were collected down wind from trees throughout a 52 h sampling period (Figure 1), however, the quantity of bacteria declined rapidly in the first few h of dispersal. The relationship between quantity of bacteria dispersed and time was described by a power law model. In a second experiment over 4 h and more frequent sampling 50% of the total bacteria dispersed were collected within the first 10 min of the wind/rain event (Table 1). The concentration of bacteria in the splash declined rapidly in the first few minutes of dispersal. Funnel samplers located under the canopy showed a similar trend in bacteria dispersal with time.

Both short- and long-term storm events occur in Florida and even the latter are often characterized by bands of fast moving, short-lived storms. These results suggest that it is during the first few minutes of a storm that greatest quantities of *Xac* bacteria are dispersed. Although bacteria continue to be produced beyond the first few minutes of the storm the concentrations are dramatically reduced, so the first minutes of a storm are likely to be a critical period for dispersal of *Xac* bacteria and infection of surrounding citrus.

Table 1. Cumulative proportion (%) of bacteria collected down wind of car infected trees over 4 h.

Time (mi	% of initial bacteria collected	Conc. (cfu/r
0	100.0	1.4 x
10	30.5	5.0 x
20	15.3	2.0 x
30	34.8	5.0 x
45	26.3	1.9 x
55	5.0	1.0 x
65	13.0	1.0 x
80	7.9	7.6 x
100	5.1	5.4 x
120	4.3	4.2 x
140	3.3	3.9 x
160	3.1	3.2 x
180	2.2	2.1 x
200	2.2	2.9 x
220	2.7	3.0 x
240	2.0	2.2 x

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P14 - Effective detection and genome sequencing approaches of *Candidatus* Liberibacter sp. causing Huanglongbing (HLB) in São Paulo, Brazil.

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Hunglongbing (HLB, or ex-greening) was first reported in Brazil in July of 2004 (Coletta-Filho et al., 2004 –Plant Disease, 88:1382). Affected citrus groves were found around Araraquara city, in São Paulo State, and in addition to the Asian form, another genetic variant was identified and named *Candidatus* Liberibacter americanus (Teixeira et al., 2005 - Plant Disease, 89:107). PCR protocols for detection of both variants were developed (Coletta-Filho et al., 2005 – Fitopatologia brasileira, 30:60) employing efficient DNA extraction and a novel set of specific primers for each variant. Single and duplex reactions were performed. This protocol has been used successfully on thousands of samples from different farmers under the HLB-State-wide-eradication-program. Symptoms of HLB on leaves are mainly unspecific when analyzed in the lab or in detached leaves. However, careful observation of different types of samples revealed high correlation of a typical leafy-blotchy symptom and positive PCR results in sweet orange leaves, thus allowing the development of a reliable visual diagnostic test (Carlos et al., 2005 - Fitopatologia brasileira, 30:60). Briefly, green regions within secondary veins are found irregularly distributed through the upper face of leaves without symmetry across the main vein. PCR and visual detection systems have been used for HLB diagnosis in the HLB-State-wide-eradication-program, which reached 65,000 samples analyzed up to date. Real time quantitative PCR system for detection both American and Asian strains of Liberibacter is been developed. Partial genome sequencing efforts have used not only symptomatic citrus leaves and *Diaphorina citri* as source of Liberibacter sp. but also alternative hosts as periwinkle infected by dodder plant.

P15 Citrus Defoliation: A Strategy to Prevent the Spread of Citrus Canker in Florida

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Citrus canker caused by *Xanthomonas axanopodis* pv *citri*, an invasive species, continues to impose adverse economic impacts to the Florida citrus industry (Graham et al. 2004). The citrus canker eradication program (CCEP) was established in 1995 to avert the spread of the disease. A 1900-foot rule (≈ 260 acres) was determined to capture the potential spread of bacteria and eradicate the disease (Gottwald et al. 2001). Increased international travel, the devastating 2004 hurricane season, legal disputes, movement of infected plant material by home owners, and other factors have made efforts to contain and eradicate the disease more difficult. In addition, the eradication campaign faced a shortage of manpower for detection and timely removal of infected and exposed trees post-2004 hurricane season. Thus, defoliation of citrus trees within the 1,900 foot rule was proposed as an alternative to help prevent the spread of the disease to non-infected groves.

In field experiments conducted on 22-year old grapefruit trees in Florida, infected with canker, a tank mix of FINISH PRO and GINSTAR EC defoliants, applied within the 1900-foot rule, at rates of 72 to 96 oz/acre and 48 to 64 oz/acre, respectively, provided more than 90% defoliation at 5 and 35 days after application (DAA). Terminal die-back was consistently observed throughout the trial. Flush and leaf regrowth was less than 5% at 35 DAA. Rapid defoliation and the prevention of tissue regrowth make the combination of FINISH and GINSTAR a powerful tool to help prevent the weather-driven spread of canker before trees are removed. A 24c label is pending approval for the tank-mix use of these two defoliants in Florida citrus groves.

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P-16 Protoplast Transformation and Regeneration of Transgenic 'Hamlin' Sweet Orange Plants Containing a cDNA Xa21 *Xanthomonas* Resistance Gene and GFP

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Citrus canker disease caused by the bacterial pathogen *Xanthomonas axonopodis* pv. *citri* is becoming a worldwide problem. *Xa21* gene is a member of the *Xa21* gene family of rice that provides broad spectrum *Xanthomonas* resistance in rice. 'Hamlin' sweet orange (*Citrus sinensis* (L.) Osbeck) is one of the leading commercial cultivars in Florida because of its high yield potential and early maturity. 'Hamlin' also has a high regeneration capacity from protoplasts and is often used in transformation experiments. Since the citrus canker pathogen is in the same genus, this gene may have potential to function against canker in citrus. The wild-type *Xa21* gene contains an intron, and there are some questions whether dicot plants can process genes containing monocot introns (the cDNA is intron free). Plasmids DNA, encoding the non-destructive selectable marker *EGFP* (Enhanced Green Fluorescent Protein) gene and the cDNA of the *Xa21* gene were transformed into 'Hamlin' orange protoplasts using polyethylene glycol. More than 200 transgenic embryoids were recovered. More than 400 transgenic plants were developed from seventy-five independent transgenic events. PCR analysis revealed the presence of the cDNA of the *Xa21* and the *GFP* genes in the transgenic plants. Some of the plants have only GFP. Southern analysis is showing integration of the cDNA into different sites ranging from 1-5 sites per plant. Real-Time PCR is showing integration of the cDNA into different sites in citrus genome ranging from 1-4 copies per plant. Western analysis is showing the expression of the cDNA of the Xa21 gene in the transgenic citrus plants. This is the first time that a gene from rice has been stably integrated and expressed in citrus plants. A citrus canker challenge assay is in progress.

P-17 Current Research on the Efficacy and Timing of Pesticide Applications for Suppression of Asian Citrus Psyllid (*Diaphorina citri*) Populations in Florida Citrus.

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Management of citrus greening disease involves removal of infected plants, production of disease-free nursery plants and management of the vector, the Asian citrus psyllid (ACP) (*Diaphorina citri* Kuwayama), by means of chemical and biological control (Halbert and Manjunath, 2004). If pesticide applications for vector control are to be used and citrus production remain profitable, products utilized must be effective, be properly timed, provide benefits in terms of reducing other pest problems that usually require pesticide applications and not create new pest problems by disrupting naturally-occurring biological control agents. Currently, few pesticides are registered for use in Florida citrus that have been shown to be effective in reducing ACP populations (Browning et al., 2005). Foliar applied products (e.g., fenpropathrin) typically provide quick knockdown of ACP when populations are observed on developing flush. Repeated application of such products should be minimized to avoid outbreaks of non-target pests and more importantly pesticide resistance. Soil-applied systemic pesticides (e.g., imidacloprid and aldicarb) take 1-2 weeks to move from the roots to the foliage and thus must be applied prior to buildup of pest populations. When applied properly, these products provide protection to the developing new flush while having little or no effect on natural enemy populations. Both imidacloprid and aldicarb have been shown to be effective for controlling ACP (and other pests) on trees less than eight feet in height. Studies are underway to determine the efficacy and duration of control of ACP by soil-applied products on large citrus trees. Such broad-spectrum systemic products might provide a multi-targeting approach to managing several citrus pests without additional pesticide applications for ACP at certain times of the year. One important issue that must be addressed prior to developing recommendations for pesticide suppression of ACP is the duration of feeding time by an infected ACP required to transmit greening disease to a healthy plant. We are currently evaluating the amount of time it takes for both systemic and foliar products to kill ACP when placed on treated plants. This information, together with more research by pathologists investigating disease transmission will provide a better understanding of how to better manage this vector-pathogen association.

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P-18 Screening citrus germplasm for resistance to *Xanthomonas axonopodis* pv. *Citri*

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Numerous studies have been conducted to identify sources of resistance to *Xanthomonas axonopodis pv. citri (Xac)* among citrus and citrus relatives (Gottwald et al., 2002 and references therein). Kumquats (*Fortunella* sp.) and Calamondins (*Citrus mitis*) have shown to be highly resistant and mandarins (*C. reticulata*) somewhat resistant. Our objective was to compare the resistance of selected *Citrus* species, citrus hybrids, with an emphasis on selections of *C. reticulata* and hybrids of *Fortunella* sp. and citrus relatives to *Xac.* Two separate experiments were conducted. The first experiment focused on determining if differences in resistance exist among 20 *C. reticulata* genotypes and included three other citrus species and citrus relatives *Glycosmis pentaphylla* and *Clausena hardimandiana*. Plants were inoculated with *Xac* strain A either by injection infiltration (Gottwald and Graham, 1992) or needle-prick. For each genotype and inoculation method there were 3 single tree replications; 4 leaves on each tree were inoculated on 3 sites. The second experiment included 10 members of the genus *Citrus* (*C. macrophylla, C. aurantium, C. excelsa, C. jambhiri, C. macroptera, C. medica, C. paradisi, C. reticulata, C. sinensis, C. aurantifolia*), 10 hybrids of *Fortunella* sp. (*[Fortunella sp. X Citrus aurantifolia]], [Citrus aurantifolia x Fortunella], [(Poncirus trifoliata x Citrus sinensis) x Fotrunella sp.]*, *[Citrange x (Fortunella x Citrus) c. calamondin]*) 5 additional citrus hybrids (*[Citrus limon x C. aurantifolia], [(C. reticulata x C. paradisi) x C. paradisi]*, *[C. limon x C. aurantifolia]*, *[Poncirus trifoliata x C. paradisi]*, *[Poncirus trifoliata x C. sinensis]*) and *Poncirus trifoliata* representing a total of 31 different selections. Plants were needle-prick inoculated with both *Xac* strain A and *Xac* strain A Wellington. There were 3 single tree replicates of each genotype. Two leaves on each plant were inoculated at 5 sites with *Xac* strain A and at 5 sites with *Xac* strain A^W (Wellington strain) (Sun et al., 2004). All inoculations were done using suspensions of *Xac* at a concentration of 10^4 cfu/mL. Trees inoculated by injection infiltration were examined 14 da, at which time the number of lesions per inoculation site were recorded. Trees inoculated by needle-prick were examined 7 and 40 da at which times lesion diameter at each inoculated site was recorded. The difference in lesion diameter between 7 da and 40 da was calculated. All data were subjected to analysis of variance. In both experiments there were highly significant differences among genotypes in response to inoculation with Xac. In the first experiment, regardless of inoculation method, *G. pentaphylla* and *C. hardimandiana* were found to be highly resistant to *Xac* whereas *C. paradisi* was least resistant. Using the needle prick method of inoculation and measuring lesion dia. there appeared to be a wider range of resistance among the *C. reticulata* selections than with the infiltration method and measuring the number of lesions per inoculation. In the second experiment for both *Xac* strain A and A^W, Chinotto sour orange, Carrizo citrange, Eustis limequat, and *P. trifoliata* were the most resistant. The Thornless key lime x Meiwa kumquat hybrids showed a range of resistance from among the most susceptible to among the most resistant. Our results expand on previous studies on resistance of citrus and citrus relatives to *Xac* and indicate that there may be potential for increasing resistance by breeding using selected parents.

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P-19 Transformation of citrus cultivars with antimicrobial genes for potential resistance to citrus canker (*Xanthomonas axonopodis* pv *citri*)

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Citrus canker has been designated as an important emerging disease in the U.S. Presently, there is limited chemical control, and no known sources of genetic resistance exist within *Citrus*. Therefore, citrus improvement depends on recombinant DNA and plant transformation methods that go beyond the present capabilities of classical breeding. We have transformed susceptible grapefruit such as 'Rio Red', 'Ruby Red', 'Duncan', and the sweet orange 'Hamlin' with the bovine lysozyme gene and the spinach defensin gene. These two genes are known to encode antimicrobial proteins, found in food, those disrupt bacterial and fungal membranes. Each gene was transformed independently using *Agrobacterium tumefaciens* strains EHA105 and C58C1. Citrus explants with transformed shoots were selected and grafted on sour orange rootstock. Stable gene integration was confirmed by histochemical staining of various tissues and Southern blot analyses. Gene transcription was confirmed by northern blot analyses, and gene expression levels were determined by western blot analyses. We will report results of preliminary experiments in which transgenic plants have been challenged by *X. axonopodis* pv *citri*.

P-20 The *hms* Locus in *Xanthomonas* spp.: a Candidate for Biofilm Formation and Virulence.

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The *hms* locus in the sequenced genome of *Xanthomonas axonopodis* pv. c*itri (Xac)*, the Asian strain of the bacterial agent responsible for citrus canker, bears remarkable homology to the *pga* locus in *Escherichia coli* K12. The established role of the *pga* locus in biofilm formation suggests a possible role for the *hms* locus as a virulence factor in *Xac*. To determine the prevalence of the *hms* locus in related phytopathogenic bacteria, *X.* v*esicatoria* (*Xv*), a causative agent of bacterial spot disease on pepper and tomato, was probed for the presence of these adhesin-related genes of the *hms* locus. With specific primers designed from the sequences of the *hmsR* and the *hmsF* genes of *Xac*, and the homologs of the *pga* locus, *pgaC* and *pgaB* in *Escherichia coli* K12, these two genes were detected in *Xv* by PCR and their nucleotide sequences were determined. The *Xv* sequence (1095/1254 nucleotides) of the *hmsR* gene showed 95% identity, by amino acid composition, to *Xac.* The *Xv* sequence (1323/1899 nucleotides) of the *hmsF* gene showed 91% identity to *Xac*. The 7 kb *hms* locus, comprised of the four genes, *hmsR*, *hmsF*, *hmsH*, and *XAC1810*, occurs in *Xac* between *XAC1808* (a homolog for genes encoding aldehyde dehyrogenase) and *fhaC* (a homolog for genes encoding hemolysin activator/secretion protein). Translated sequences of XAC1808 and *fhaC* in *Xac* showed 98% identities with partial sequences of homologs in *Xv*. Detection of *hmsR* and *hmsF* in *Xv* indicated a significant degree of synteny with *Xac* for this region of the genome. The *hms* locus has also been identified in the sequenced genome of *Xanthomonas oryzae*, although its location relative to other genetic loci support a greater mobility for this locus. A deletion in the *hmsR* locus in *X. axonopodis* pv *citri* results in a decrease in disease severity on grapefruit. The presence of the *hms* locus in pathovars of *X. citri*, *X. oryzae*, and *X. vesicatoria*, and its distinctive homology to the *pga* locus in *E. coli*, suggests a role for poly-B-1,6-N-acetyl-D-glucosamine as an adhesin and potential virulence factor in these *Xanthomonas* spp.

P-21 Progress Towards the Development of an Effective Risk Analysis Process for the Florida Citrus Nursery Industry to Mitigate the Impact of Citrus Canker and Huanglongbing

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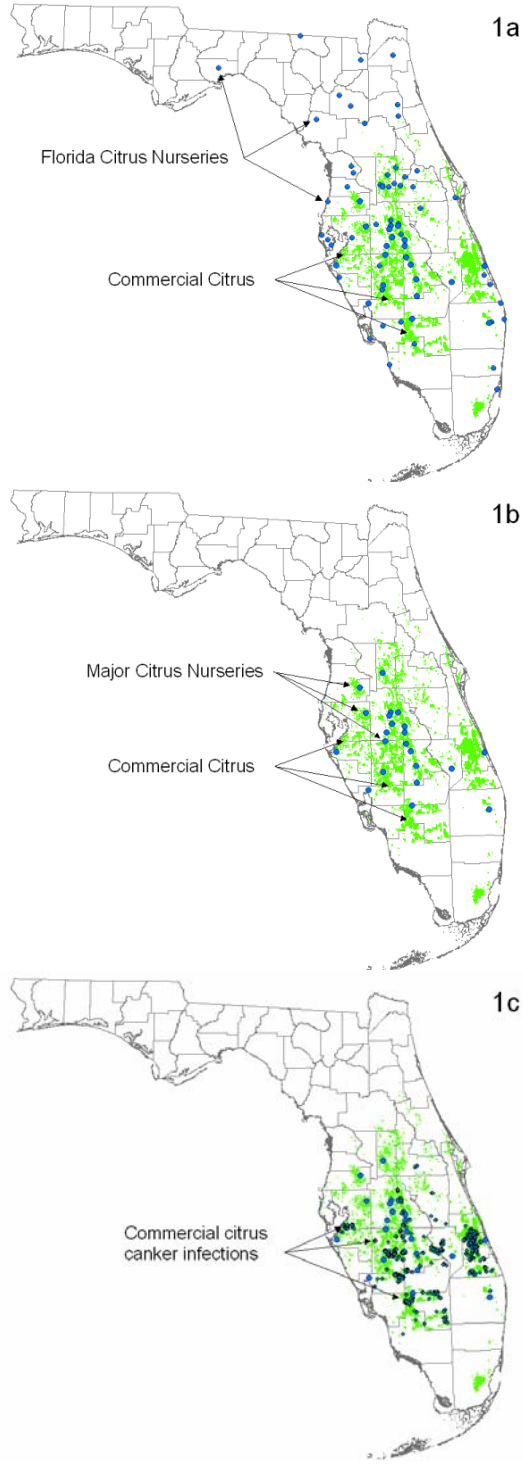
The citrus industry within the state of Florida is being directly impacted by two recently introduced bacterial diseases, citrus canker and huanglongbing. As the commercial citrus industry is trying to develop efficient strategies to eradicate, control, or manage the diseases, it is becoming readily apparent that the citrus nursery industry must play a major role in any control program that is developed to manage these two diseases. Although on the surface it appears that the citrus nurseries are widely distributed across the state (Figure 1a), the largest nurseries that supply the majority of the trees for the industry are largely concentrated in the same areas as the majority of the commercial groves (Figure 1b). As a result of the increasing frequency of detection of citrus canker in close proximity to the commercial nurseries (Figure 1c), and with the recent detection of huanglongbing in at least six citrus growing counties within the state, many commercial groves and citrus nursery operations are trying to decide if new, more isolated, nursery sites should be established. However, currently there are no guidelines established for evaluating the existing sites or potential new sites with respect to the suitability of the sites to provide disease-free trees to the industry.

In a joint effort with commercial grove production managers, commercial nursery owners, and with input from researchers, a spreadsheet-based risk assessment system is being developed that takes into account risk factors associated primarily with nursery site location and construction. Among the factors being considered are proximity to commercial and residential citrus, inoculum density potential, proximity to commercial packing/processing plants, proximity to international points of entry, proximity to the costal regions, construction of greenhouse facilities, sources of budwood, presence and type of windbreaks, presence of alternate hosts of the diseases and insect vectors, and other factors related to citrus nursery production and disease avoidance.

Figure 1. Location of Florida citrus nurseries in relation to the commercial industry and citrus canker finds within the last 2 years



P-22 Incidence of *Diaphorina citri* (Hemiptera: Psyllidae) and its natural enemies in Puerto Rico and Florida

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The Asian citrus psyllid (ACP) is well established in Puerto Rico, mostly on *Murraya paniculata*. A year-long survey of *D. citri* populations in citrus at 4 research stations in Puerto Rico showed that ACP is spatially and temporally patchy in the citrus habitats on the island. The parasitic wasp *Tamarixia radiata* (Hymenoptera: Eulophidae) is found throughout the year, even during times of low citrus flush activity and low psyllid numbers. Percent parasitism fluctuated from 40 to 100% with no clear seasonal patterns. A study laboratory of coccinellid feeding habits demonstrated the potential of ladybeetles to impact ACP numbers in Puerto Rico although aphid predators continue to dominate. *Tamarixia radiata* caused almost 100% mortality of *D. citri* in an open screenhouse in Rio Piedras, but parasitism was negligible in a coastal field station during summer. However, psyllid mortality remained high due to predation on young instars, primarily from ants. Predation of young instars by coccinellids was the dominant mortality factor during summer in Florida with parasitism by *T. radiata* becoming evident in fall.

P-23 Contribution of Predation and Parasitism to Mortality of Citrus Leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) Populations in Florida

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Direct observation and exclusion methods were used to evaluate the individual contributions of predation and parasitism on CLM mortality in the field. Ant predation was the largest single cause of mortality, accounting for over 30% of all deaths by natural enemies, and 60% of all death by predators. Early (1st and 2nd stage) instars were most subject to ant predation. *Ageniaspis citricola* was the most important parasitoid contributing 8.2-28.6% mortality compared to 9.6-14.7% from indigenous parasitoids. The total impact of biotic mortality on CLM populations was 65-70%.

P-24 Reducing Canker Risk through Biological Control of Citrus Leafminer

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Citrus leafminer, *Phyllocnistis citrella* is a know risk factor in the spread of citrus canker. Wounded cuticle caused by passage of the leafminer through the epidermis allows entry of bacteria with no new cuticle formation for up to 2 weeks during which time the leaf is especially susceptible to canker. Furthermore, spread within mine results in increased inoculum available for spread. Dramatic acceleration of canker spread was seen in Brazil after introduction of CLM in 1996. Inability eradicate canker compared to previous outbreaks and high incidence of canker in less susceptible varieties are further indications of the role of CLM in facilitating spread of canker. Mitigation of canker spread by biological control was demonstrated following the introduction of *A. citricola* in Brazil. Predation mostly by ants is greatest mortality factor on CLM populations in SW Florida, followed by parasitism, mostly by *Ageniaspis citricola* during the humid summer. However, *A. citricola* is unable to control CLM during the dry spring. A drought resistant parasitoid is required to compliment *A. citricola* under these conditions. *Citrostichus phyllocnistoides* attacks young instar CLM larvae and has reduced infestations to non-economic levels in Spain and other Mediterranean countries under droughty conditions. This parasitoid is currently being reared in Florida under quarantine and should be permitted for release this spring to reduce CLM populations and thus the rate of canker spread.

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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**

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Orlando, FL

Research Recommendations: Huanglongbing 2005

- 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

T.R. Gottwald, J. Graham, W.N., Dixon, A. LaVigne and P. Berger. 2005. Proceedings of the 2nd International Citrus Canker and Huanglongbing Research Workshop. Orlando, FL.

2nd International Citrus Canker and Huanglongbing Research Workshop
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Research Recommendations: Huanglongbing 2005

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

Research Recommendations: Huanglongbing 2005

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. alernative hosts

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

- 4 Characterization Taxonomy of HLB
  - 4.1 Differentiation of species and strains
  - 4.2 Genetic diversity studies
  - 4.3 Repositories for voucher specimens

- 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

- 6 Culturing HLB
  - 6.1 Culturing HLB

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Research Recommendations: Huanglongbing 2005

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

- 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

Research Recommendations: Huanglongbing 2005

- 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

- 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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Research Recommendations: Huanglongbing 2005

- 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

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
- 12.4Genetic modification of vector
- 12.5 Development of transformation methodologies for citrus and citrus relatives and ornamentals

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Research Recommendations: Huanglongbing 2005

- 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

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

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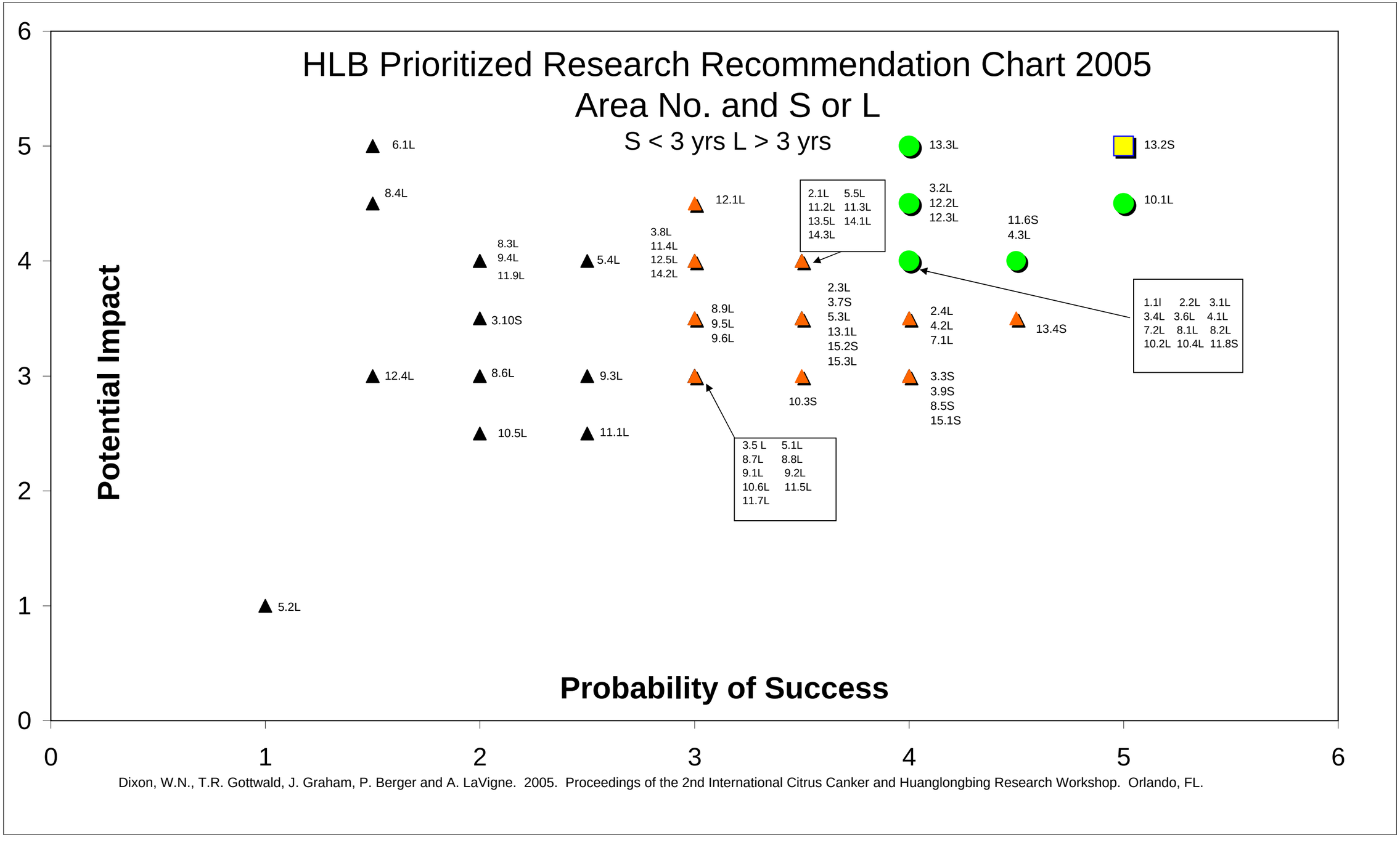
Research Recommendations: Huanglongbing 2005

- 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



Dixon, W.N., T.R. Gottwald, J. Graham, P. Berger and A. LaVigne. 2005. Proceedings of the 2nd International Citrus Canker and Huanglongbing Research Workshop. Orlando, FL.

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	**Economics**				
	1.1		x	4	4
2	**Alternative Hosts**				
	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	**Detection of Disease and Vector**				
	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	**Characterization and Taxonomy**				
	4.1		x	4	4
	4.2		x	4	3.5
	4.3		x	4.5	4
5	**Resistance and Breeding**				
	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	**Culturing HLB**				
	6.1		x	1.5	5
7	**Pathogens/Vector/Host Interactions**				
	7.1		x	4	3.5
	7.2		x	4	4
8	**Chemical Control**				
	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	**Biological Control**				
	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	**Cultural Control**				
	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	**Epidemiology**				
	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	**Transgenics**				
	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	Genomics				
	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	Vector Biology				
	14.1		x	3.5	4
	14.2		x	3	4
	14.3		x	3.5	4
15	Fruit Yield and Quality				
	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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Research Recommendations: Citrus Canker 2005

- 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 Antangonistic Effects of HLB Controls

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Research Recommendations: Citrus Canker 2005

- 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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Research Recommendations: Citrus Canker 2005

- 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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Research Recommendations: Citrus Canker 2005

- 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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Research Recommendations: Citrus Canker 2005

- 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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Research Recommendations: Citrus Canker 2005

- 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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Research Recommendations: Citrus Canker 2005

- 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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Research Recommendations: Citrus Canker 2005

- 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

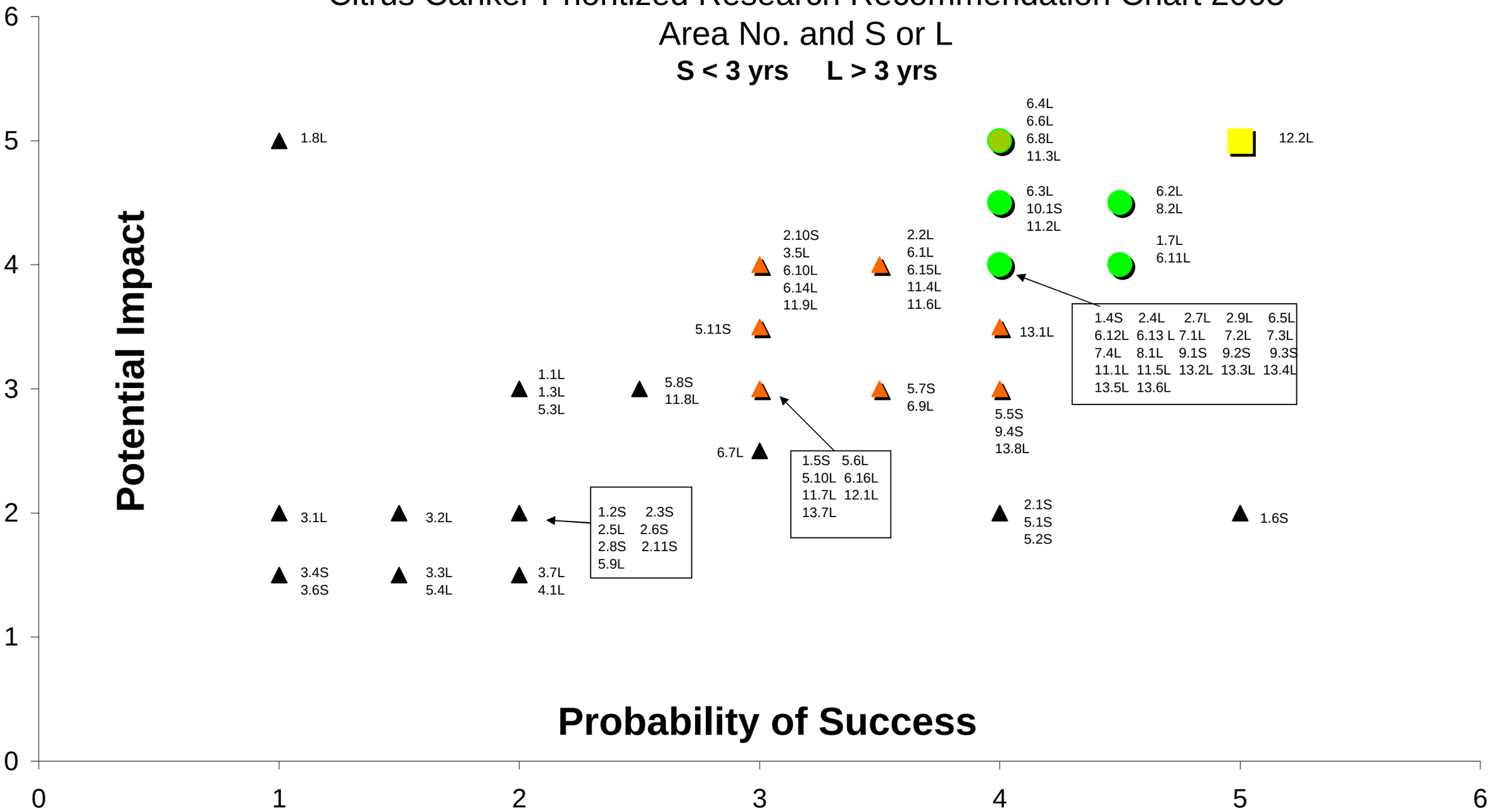
- Eradication Campaign – Epidemic
- Management – Endemic: surrogate organisms, environmental variations
- Application and impact of windbreaks, defoliation techniques
- Pre-eradication inoculum suppression techniques – defoliation, tarping

- 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



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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	**Chemical**				
	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	**Cultural Control**				
	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	**Biological Control**				
	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	**Remote Sensing**				
	4.1		x	2	1.5
	4.2		x	1.5	2
5	**Detection**				
	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	**Resistance**				
	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	**Differentiation**				
	7.1		x	4	4
	7.2		x	4	4
	7.3		x	4	4
	7.4		x	4	4
8	**Pathogenesis**				
	8.1		x	4	4
	8.2		x	4.5	4.5
9	**Survival**				
	9.1	x		4	4
	9.2	x		4	4
	9.3	x		4	4
	9.4	x		4	3
10	**Economics**				
	10.1	x		4	4.5
11	**Transgenics**				
	111		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	**Genomics**				
	12.1		x	3	3
	12.2		x	5	5
13	**Epidemiology**				
	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

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