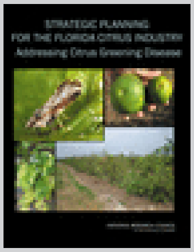

Strategic Planning for the Florida Citrus Industry: Addressing Citrus Greening

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FOR THE FLORIDA CITRUS INDUSTRY
Addressing Citrus Greening Disease

Committee on the Strategic Planning for the Florida Citrus Industry: Addressing Citrus Greening Disease (Huanglongbing)

Board on Agriculture and Natural Resources

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by Elaine A Backus, U. S. Department of Agriculture. Appointed by the National Research Council she was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

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Abbreviations and Acronyms

ACP	Asian citrus psyllid
ABP	anti bacterial peptide
ACPS	advanced citrus production system
AFRI	Agriculture and Food Research Initiative
APHIS	Animal and Plant Health Inspection Service (USDA)
ARS	Agricultural Research Service (USDA)
CAC	Citrus Administrative Committee
CC (or CBC)	citrus canker, citrus bacterial canker
CCTF	Core Citrus Transformation Facility
CDFA	California Department of Food and Agriculture
cDNA	complementary DNA
CHMA	Citrus Health Management Areas
CHRP	Citrus Health Response Program
CiLV	bacilliform virus (causal agent of Leprosis)
*C*Laf	*Candidatus* Liberibacter africanus
*C*Lam	*Candidatus* Liberibacter americanus
*C*Las	*Candidatus* Liberibacter asiaticus
c-PCR	competitive polymerase chain reaction
CPHST	Center for Plant Health Science and Technology
CRDF	Citrus Research and Development Foundation
CREC	Citrus Research and Education Center
CSD	citrus sudden death
CTAB	cetyltrimethylammonium bromide
CTV	citrus tristeza virus
CVC	citrus variegated chlorosis
DEP	Department of Environmental Protection
DNA	deoxyribonucleic acid
DMDS	dimethyl disulfide
DMS	Differential Mobility Spectrometer
DOD	US Department of Defense
DOE	US Department of Energy
DOL	US Department of Labor
DPI	Division of Plant Industry (FDACS)
dsRNA	double stranded RNA

ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
EPA	US Environmental Protection Agency
EST	expressed sequence tag
FCIRCC	Florida Citrus Industry Research Coordinating Council
FCM	Florida Citrus Mutual
FCP	Florida Citrus Packers
FCPA	Florida Citrus Processors Association
FCPMA	Florida Citrus Production Managers Association
FCPRAC	Florida Citrus Production Research Advisory Council
FDA	US Food and Drug Administration
FDACS	Florida Department of Agriculture and Consumer Services
FDOC	Florida Department of Citrus/Florida Citrus Commission
FFSP	Florida Foundation Seed Producers, Inc.
FGFSA	Florida Gift Fruit Shippers Association
FIFRA	Federal Insecticide Fungicide and Rodenticide Act
FL DEP	Florida Department of Environmental Protection
FNGLA	Florida Nursery, Growers and Landscape Association
GC	guanine-cytosine
GWSS	glassy winged sharpshooter
HIPREE	Huanglongbing Information Platform for Research, Extension and Education
HLB	huanglongbing
ICE	US Immigration and Customs Enforcement
IFAS	Institute of Food and Agricultural Sciences (UF)
ICGC	International Citrus Genome Consortium
IOCV	International Organization of Citrus Virologists
IP	intellectual property
IPM	integrated pest management
IR-4	Interregional Project Number 4
IRM	insecticide resistance management programs
IRREC	Indian River Research and Education Center
LFM	lateral flow microarray
MA	monoclonal antibodies
Mbp	megabase pairs
MCDV	maize chlorotic dwarf virus
MDMV	maize dwarf mosaic virus
miRNA	micro RNA

NAE	National Academy of Engineering
NIH	National Institutes of Health
n-PCR	nested polymerase chain reaction
NSF	National Science Foundation
NVDMC	New Varieties Development and Management Corporation
OHS	Open Hydrophonics System
OMRI	Organic Materials Review Institute
OSHA	US Occupational Safety and Health Administration
OSU	Ohio State University
PASS	potentially actionable suspect samples
PCR	polymerase chain reaction
PC-STR	psyllid control and symptomatic tree removal
PFD	postbloom fruit drop
PI	principal investigator
PIPE	Pest Information Platform for Extension and Education
PIPRA	Public Intellectual Property Resource for Agriculture
PMAP	Pest Management Alternatives Program
PPQ	Plant Pest and Quarantine
(+) ssRNA	positive, single stranded RNA
q-PCR	quantitative real-time polymerase chain reaction
rDNA	ribosomal DNA
R&D	research and development
RFP	Request for Proposals
RNA	ribonucleic acid
RNAi	RNA interference
rRNA	ribosomal RNA
RTi-PCR	real-time polymerase chain reaction
SAB	scientific advisory board
SAR	systemic acquired resistance
SCRA	Specialty Crops Regulatory Assistance
SGDL	Southern Gardens Diagnostic Laboratory
SIT	sterile insect technique
SNP	single nucleotide polyphormism
SPS	Saõ Paulo State
SWFREC	Southwest Florida Research and Education Center
TEM	transmission electron microscopy
TIG	Technology Innovation Group, Inc.
tRNA	transfer RNA

UAE	United Arab Emirates
UC	University of California
UF	University of Florida
USDA	US Department of Agriculture
USPTO	US Patent and Trademark Office
WBDL	witches' broom disease of limes
VOC	volatile organic compound

Summary

Among the many diseases of citrus that have invaded or could invade Florida, greening represents the greatest threat to the industry. Greening has been known internationally, from its first description in the early 1900s in China, as huanglongbing (HLB, translated as "yellow shoot disease"). In Florida, HLB is associated specifically with the bacterium *Candidatus* Liberibacter asiaticus (*C*Las), the presumed causal agent. The insect vector, the Asian citrus psyllid (ACP, *Diaphorina citri*) acquires *C*Las by feeding on the nutrients carried by phloem cells in the citrus plant and injects the bacterium to other citrus plants. ACP was first detected in Florida in 1998. Shortly thereafter, ACP had spread to the point that eradication became unconceivable. HLB itself was discovered in Florida in August of 2005. HLB is now present in the 34 citrus-producing counties of Florida, but is most prevalent in the southern areas of the state.

In April 2009, the National Research Council, at the request of the Florida Department of Citrus (FDOC), formed the Committee on the Strategic Planning for the Florida Citrus Industry: Addressing Citrus Greening Disease (Huanglongbing). In developing a strategic plan, the committee was charged to examine: 1) the current citrus disease situation in Florida and the status of public and private efforts to address citrus greening and other diseases; including lessons learned; 2) the capacity of the industry to mobilize a scientifically based response to current disease threats and to translate scientific advances into products and services for the protection of Florida citrus industry in the short and long term, and 3) the relationship of the industry to public, academic, and private research, and to regulatory and funding organizations at the state and federal level, with respect to controlling citrus greening and developing a comprehensive solution to citrus greening and other diseases. In the course of its deliberations, the committee made the following key findings.

KEY FINDINGS

1. Citrus products are generally appreciated as being both desirable and healthful, and this appreciation is justified. Without Florida citrus production, orange juice and other citrus products in our stores would be fewer, less diverse, possibly of reduced quality, and likely more expensive.

2. Citrus is emblematic of Florida. There would be great repercussions for Florida's economy if the estimated $9.3 B annual economic benefit of the citrus industry were to be lost or significantly diminished. Florida's citrus is an industry very worth preserving.

3. HLB and HLB control measures reduced Florida orange juice production by several percent by 2008, and losses will likely increase, at least in the immediate future and possibly longer.

4. The two major parts of the Florida citrus industry are the larger juice processing sector and the more profitable, per acre, fresh market sector. The incompletely defined dynamic between citrus production and the processing plants is such that when plants operate below their most productive level, profits are threatened. If a plant closes, producers suffer reduced marketability of their oranges. Thus, declining production can induce a downward spiral.

5. In the Florida situation, HLB is entirely correlated with the presence of, and is presumed to require the participation of, *C*Las and its insect vector ACP.

6. In Brazil and in Florida, rigorous three-pronged programs have had a demonstrated saving effect in areas not yet severely affected by HLB. The programs rely on (i) production of citrus propagation materials in insect-proof facilities, which has been mandatory in Florida nurseries since January, 2008, (ii) strong reduction of the ACP population, and (iii) identification and removal of infected citrus trees, the principal reservoir of *C*Las. Although these programs have reduced the percentage of infected trees in some areas, the numbers of infected citrus trees in Florida as a whole continues to increase.

7. Increased use of insecticide sprays, as currently required for successful suppression of ACP populations, brings with it risks of ACP developing resistance to one or more of the most useful insecticides and of adverse affects on beneficial insects. More information on ACP behavior and HLB ecology and new approaches are needed to improve ACP suppression.

8. The identification and removal of *C*Las-infected citrus trees is currently dependent on scouting for visible HLB symptoms. The process is expensive, not sustainable for many orchards, and very likely would be greatly improved if infected but as yet asymptomatic trees could be identified.

9. The most powerful long-term HLB management tool likely will be citrus cultivars resistant to *C*Las and preferably to ACP as well. However, there is no clear path by conventional breeding to deliver a robust resistant citrus for many commercial species because these species lack known sources of resistance and a facile breeding system.

10. It is likely that the breeding systems for sweet orange and some other citrus can be greatly enhanced in the long run by capabilities derived from genome sequence analysis and other technologies.

11. Genetic engineering, in the form of transgenic citrus or citrus inoculated with a transgene-expressing virus vector, holds the greatest hope for generating citrus cultivars resistant to *C*Las and ACP.

12. New information on *C*Las, ACP and citrus, and the advances of modern biology and chemistry in general, suggest new research directions that may reveal new strategies for HLB mitigation.

13. The citrus industry and various government agencies have made very significant investments in research on HLB and approaches to HLB mitigation. Results usable in effective HLB mitigation will probably be derived more rapidly from research projects if efforts are more unified than they are at present, on the national and international level, and there is more emphasis on strategic planning. When (or if) HLB no longer is a significant threat, an integrated research infrastructure would continue to serve the industry well.

The Florida Citrus Industry

The favorable conditions for citrus production in Florida have allowed the industry to grow to the point of having a $9.3 billion annual economic impact on the state. Throughout the Florida citrus industry's history, this success has been tempered and defined by the adverse effects of the natural calamities of freezes, hurricanes and diseases, and, in more recent times, by urbanization, international competition, and shortage of water.

Due to the Great Freeze of 1894–1895, citrus production was abandoned in northern Florida and in other southeastern US states and has become concentrated in mid- and south-Florida. The Florida freezes of the 1960s provided an opening for the expansion of the Brazilian citrus production. Brazil now accounts for about 37 percent of the worldwide citrus production, whereas the United States' share is about 17 percent. In 2004–2005, hurricanes were largely responsible for the reduction in sweet orange (38 percent) and grapefruit (69 percent) production compared to the year before. Urbanization has displaced citrus production from most of Florida's coastal areas, leaving the majority of the production in the more freeze-prone inland areas. The seasonality of Florida rainfall means that virtually all Florida citrus is irrigated. A more subtle factor influencing the citrus industry is the interaction between production and processing, as indicated in *Key Finding 4*, above.

Citrus Greening (Huanglongbing) and its Florida Vector, Asian Citrus Psyllid

The first observations of diseased citrus corresponding to what is now recognized as HLB were in southern China in the late 19th century. In the mid-20th century, the Chinese researcher K.H. Lin provided a scientific description of HLB and demonstrated its infectious nature. At present, it is estimated that nearly 100 million trees in 40 countries are affected by HLB. In the 1960s–1980s, HLB devastated citrus production in the Philippines, Indonesia, Thailand, and South Africa. In March 2004, HLB was recognized for the first time in the Americas in São Paulo State, Brazil. Subsequently, nearly 3 million HLB-affected sweet orange trees were removed in Brazil. HLB was found in Florida in 2005. In North America, HLB now occurs in Cuba, Belize, Florida, Georgia, Lousiana, South Carolina, and Eastern Yucatan, Jalisco, and Nayarit, Mexico.

HLB enters a geographical region in an introduced psyllid vector or in infected live plant material. In the field, psyllid transmission (acquisition, retention through a latent period, then inoculation) is the primary mode of HLB spread. Thus, introduction and establishment of a vector species, which in Florida is ACP , always precedes establishment of HLB. In the principal mode of plant-to-plant transmission with an established psyllid population, older immatures acquire the bacteria, which circulate and propagate in the insects' bodies while they complete development to adulthood; then adult psyllids inoculate healthy citrus during feeding. It is also possible for adults to acquire the bacteria.

When HLB enters a region hitherto free of the disease, young trees show symptoms earlier and are more severely affected than mature trees. The symptoms of HLB include the appearance of yellow shoots and a blotchy mottle of the leaves. First symptoms may appear in 6–18 months. Severe symptoms may appear as early as 6 months post infection in young trees but more typically at 1–5 years, especially for mature trees. The disease progress in the orchard can be relatively fast, reaching an incidence of more than 95 percent in 3 to 13 years after onset of the first symptoms. Yield per tree is reduced and fruits of affected branches may have color inversion or be abnormally small and lopsided and have aborted seeds. “Off” flavor and small size could cause the fruit to be rejected even for juice production. There are no known citrus species, varieties or combinations of scion and rootstock that are immune to HLB.

The Causal Agent of Huanglongbing

Almost all HLB-affected citrus trees worldwide are infected with at least one of three recognized bacteria of the genus *Liberibacter*. In a few cases, HLB symptoms have been associated with phytoplasmas and not Liberibacters, in São Paulo State and China. However, in Florida, the universal association of *C*Las and ACP with HLB erased virtually all doubt about whether *C*Las should be a prime subject of research aimed at mitigating HLB. Recently, the complete genome sequence of *C*Las has been obtained by using deoxyribonucleic acid (DNA) extracted from a psyllid and DNA from infected plants.

Detection and Mitigation of Huanglongbing

Thermotherapy, shoot-tip grafting or antibiotic treatment can rid budwood of *C*Las, but antibiotic treatment of orchard trees has not proven to be a practical control method. Vigorous pruning of symptomatic parts of the tree has given only short term relief, if any. Nutritional sprays intended to suppress HLB symptoms have been used in some orchards. However, to date, controlled comparisons have not demonstrated an improvement in crop yield or fruit quality or any long term amelioration of symptoms or reduction in *C*Las titer from these treatments, and their use can diminish the effectiveness of mitigation measures based on removing sources of inoculum. In the absence of curative treatments or resistant citrus lines, the available method for coping with HLB once the disease has been discovered in a region is the 3-pronged appoach of *Key Finding 6*. Production of healthy citrus trees in “closed,” insect-proof nurseries is established practice in Florida. The other two prongs of current HLB mitigation are reducing inoculum sources and suppressing ACP.

RECOMMENDATIONS

Shaped by the key findings and its study of scientific opportunities to addresss HLB, the committee’s strategic plan consists of 23 recommendations, listed in Box S-1, and grouped according to objective: Organizational (O); Informational (In); Near- to Intermediate-Term Research and Technology (NI); and Long-Term Research and Technology (L). The recommendations are listed in approximate order of importance within each group. However, the success of some recommendations is dependent on other recommendations, requiring coordination in their implementation.

BOX S-1
RECOMMENDATIONS

Organizational (O) Recommendations

O-1. Create "Citrus Health Management Areas" in Florida.
O-2. Identify one organization and empower it to have oversight responsibility over huanglongbing (HLB) research and development efforts.
O-3. Create a centralized HLB website and data bank that is accessible to researchers and the public.
O-4. Commission an analysis of the economics of the citrus industry's responses to HLB.
O-5. Organize an enhanced annual international symposium on all aspects of HLB.

Informational (In) Recommendations

In-1. Expand extension efforts emphasizing the importance to HLB management of removing infected trees from groves.
In-2. Encourage homeowners to remove and properly dispose of backyard citrus trees, particularly HLB-affected trees.
In-3. Communicate information on HLB and its potential economic impact to government officials at the federal, state, county, and city level.

Research and Technology Recommendations: Near- to Intermediate-Term (NI)

NI-1. Improve insecticide-based management of Asian citrus psyllid (ACP).
NI-2. Support searches for biomarkers that may be exploited to detect *C*Las-infected citrus.
NI-3. Establish citrus orchard test plots for evaluation of new scouting and therapeutic methods.
NI-4. Accelerate the sequencing, assembly, annotation and exploitation of a sweet orange genome to provide a powerful tool for all future citrus improvement research.
NI-5. Support development of HLB model systems.
NI-6. Exploit the *C*Las genome sequence for new strategies of HLB mitigation.
NI-7. Support research aimed at developing alternative ACP management strategies.
NI-8. Support small-scale studies on the feasibility of alternative horticultural systems suited to endemic HLB.
NI-9. Support demonstration of RNA interference (RNAi) effects for possible suppression of ACP.
NI-10. Develop *in vitro* culture techniques for *C*las to facilitate experimental manipulation of the bacterium for insights into gene function.
NI-11. Sequence, assemble and annotate the ACP genome to provide basis for new approaches to ACP management.

continued

Research and Technology Recommendations: Long-Term (L)

L-1. Support development of transgenic HLB-resistant and ACP-resistant citrus.
L-2. Support development and testing of bactericides, therapeutics or SAR activators.
L-3. Support analysis of ACP behavior, ACP-plant interactions and ecology to enhance the knowledge base available for new ACP management strategies.
L-4. Explore possible control strategies based on release of modified psyllid males.

The scientific basis for each recommendation and its synergy with others are discussed in detail in Chapter 4 of the full report. Near- to Intermediate-Term (NI) research and technology projects are those that can generate proof-of-principle or demonstrate HLB mitigation in less than 5 years. They are most important for the current management of HLB and need to be pursued in conjunction with Organizational (O) and Informational (In) approaches. NI research also can contribute essential knowledge for Long-Term (L) research and technology, which will take more than 5 years to develop, but that offer the greatest opportunity for succesful long-term management.

In the sections that follow, several of the key recommendations are broadly discussed (not necessarily in the order presented in Box S-1) in the context of their general roles in the overall strategy, namely: achieving an organized response to HLB, addressing the current, urgent needs of the citrus industry, and developing the technology to sustain the industry in the future. Individual research and technology projects that support these roles are further described in the section on Essential Research Directions.

Responding to the Huanglongbing Threat

Industry and government both have responded to the HLB threat by significantly increasing the research effort. Citrus research has been funded by several national, regional, state, and citrus industry organizations. Following the detection of HLB in Florida, the Florida Citrus Production Research Advisory Council (FCPRAC), which directed funds to research through competitive grants program, dedicated nearly all of its funds to HLB and canker research. In 2007–2008, the FCPRAC, the state of Florida, and the FDOC, spent $1.5, $3.5, and $2 million, respectively, for HLB and canker research. In June of 2008, the newly launched Florida Citrus Advanced Technology Program (FCATP) issued an Request for Proposal (RFP) which resulted in 205 proposals. These proposals were reviewed by National Research Council-appointed panels. The FCATP awarded over $16M to the funded projects. Currently, the management of industry-connected competitive research grants program is the responsibility of the newly formed Citrus Research and Development Foundation (CRDF). An important goal is improved coordination of research funding and research project monitoring (*Recommendation O-2*).

Chapter 5 of the full report provides an extensive explanation and comparison of four models for support of research and development that may be useful in considering how to fund the needed research. These are research grants, sponsored research, contract research, and inducement prizes. Each of these models has advantages and disadvantages for advancing particular research or development project.

The incursion of HLB has been more effective than any prior event in bringing industry, government, and universities together in the defense of citrus production in Florida. Local, state, and international meetings have been organized. Attendance at such meetings has been excellent, and growers have had numerous opportunities to become current on HLB and recommended practices. Information transfer can be further enhanced by increased use of website-accessible data banks (*Recommendation O-3*) and an annual international research meeting strategically planned to maximize the possibilities for synergistic interactions among research groups and information transfer among stakeholders (*Recommendation O-5*).

Various advisors hold differing views on the best management practices for HLB. There has not always been a consensus, particularly with regard to the urgency of efforts in discovery and removal of HLB-infected trees. Nonetheless, there is a general recognition of the need to develop regulations that meet the needs of the entire industry without excessively burdening any segment. The Citrus Health Response Program (CHRP) was initiated by the USDA and FDACS, with considerable input from the University of Florida and industry groups. Although aspects of CHRP regulations were considered to be onerous, especially for the nursery industry, the current system of production of all citrus trees in screened enclosures is one result of this effort that has benefited everyone in the industry.

A more complete understanding of the already realized and the potential economic impacts of HLB, and of alternative approaches to dealing with the HLB problem, is needed. More complete knowledge of economic impacts will allow fine-tuning of research priorities for the urgent task of keeping the citrus industry viable, while new approaches to longer term HLB mitigation are being advanced (*Recommendations O-4* and *In-3*).

Actions Needed to Enhance the Current Management of Huanglongbing in Florida

There is reason to be optimistic about the prospects of a future Florida citrus industry that will rely on sustainable methods for HLB mitigation not available at present, but likely to be developed using the concepts and technology of modern biology. In the meantime, *Recommendations O-1*, *In-1*, *In-2* and *NI-1* have a high priority because of their potential for sustaining production until the to-be-developed approaches can be implemented. Citrus Health Management Areas (*Recommendation O-1*) should be created to facilitate and coordinate control of ACP and removal of affected trees, both in commercial orchards and in residential areas (*Recommendations In-1* and *In-2*). These areas are envisioned to be 10,000–50,000 acres consisting of regions with similar levels of HLB incidence. Each management area would be charged with dealing with threats to citrus production by mandating best management practices for clean-up of abandoned HLB-affected orchards, designing and implementing compensation plans, coordinating area-wide psyllid sprays, and reducing risk from infected urban citrus. Area-wide insect control strategies should utilize “window” strategies where only certain classes of insecticides are permitted during specific periods to minimize the development of resistance. *Recommendation NI-1* is intended to empower *Recommendation O-1* by promoting more integrated efforts to improve the practices for insecticide control of ACP: (i) improving the surveillance methods for ACP, (ii) registering new insecticides and developing new insecticide application protols, (iii) developing pesticide application protocols informed by ACP behavioral ecology and ACP *C*Las transmission biology, and (iv) coordinating cultural practices with insecticide applications.

In addition, *Recommendations NI-2* (identifying biological markers of infection) and *NI-3* (creating test plots to validate biomarkers and new treatments) have the potential to greatly improve the effectiveness of efforts under *Recommendation O-1*. The new approaches would incorporate biomarkers specific for *C*Las infection and instrumentation designed to efficiently detect those biomarkers, e.g., plant volatiles analyzers, optical tree analyzers, plant tissue-inserted electrochemical probes. These and other research approaches that can enhance HLB management in the short-term are discussed in the Summary section on Essential Research Directions.

Actions Needed for the Sustainable Management of Huanglongbing in the Future

The consensus approach to the long-term management of HLB is to deploy citrus lines resistant to *C*Las and ACP, which can be accomplished in principle by: (i) classical genetics and conventional plant breeding, (ii) genetic transformation to produce transgenic plants, or (iii) introduction of a bacteria- or virus-derived DNA vector bearing a resistance gene of interest. At this time, conventional plant breeding is unlikely to deliver resistant varieties because many commercial citrus species lack a functional breeding system and no genetically compatible source of resistance has been found. This situation renders genetic engineering (options ii and iii) as more viable for developing citrus with resistance to *C*Las and/or to ACP (*Recommendation L-1*). A variety of research projects can support achieving this long-term goal, such as completing the sequencing of the sweet orange genome (*Recommendation NI-4*), the genomes of *C*Las and ACP (*Recommendations NI-6 and NI-11*), and exploring the potential to use RNA interference to suppress the psyllid (*Recommendation NI-9*). These are elaborated further in the section on Essential Research Directions.

Should HLB become unmanageable in Florida with current production approaches, it will be necessary to grow citrus differently in the future. *Recommendation NI-8* encourages the exploration of new advanced citrus production systems employing high density plantings or screen houses or both, These production systems may be able to reduce infection rates or compress and enhance the citrus production cycle so that economic return can be realized before losses to HLB dominate. The economic viability and manageability of these systems over the long term also need to be investigated (*Recommendation O-4*).

ESSENTIAL RESEARCH DIRECTIONS

Discussed below are some of the Committee's specific recommendations aimed at enhancing the response to HLB and improving the mitigation of HLB through the use of various scientific approaches and technologies.

Improving Detection of Huanglongbing

Detection of *C*Las-infected trees for removal is based on visual scouting for HLB symptoms and discovers only symptomatic trees. Often the presence of *C*Las is confirmed by polymerase chain reaction (PCR) analysis. HLB-infected trees are pulled or cut down, followed by stump treatment to prevent the appearance of infected shoots. If more than a few percent of the trees in a grove must be removed each year, production will not be sustainable, at least under present conditions of citrus culture.

If *C*Las-infected trees can be detected and removed sooner than visual scouting allows, the numbers of inoculative ACP likely will be reduced significantly due to reduced period of ACP access to infected trees, fewer infected trees and parts of trees, and reduced average *C*Las titer in the trees. Biomarkers are changes in biochemical, physiological or physical properties of an organism that are indicative of specific biologic states, e.g., asymptomatic *C*Las infection. *Recommendations NI-2* and *NI-3* promote a multidisciplinary search for *C*Las-infection biomarkers in citrus. Biomarkers may be discovered (i) by direct analyses for changes in the prevalence of specific nucleic acids, proteins, other macromolecules or small molecules, including tree volatiles, or in tree optical signals, or (ii) by bioinformatic analysis of changes in gene expression, to predict infection-specific metabolic alterations.

Improving Suppression of Asian Citrus Psyllid Populations

Several factors challenge efforts to suppress ACP populations in Florida. Compared to temperate climate areas, Florida's sub-tropical climate allows more generations of insects to be produced and hence requires more intensive management practices. Citrus is a perennial crop, and *C*Las multiplies in ACP, which can then efficiently inoculate the bacterium for life.

Can biological control of ACP work to reduce HLB disease progress? ACP in Florida is attacked by many generalist predators and a number of hymenopterous parasitoids but most effectively by coccinellid beetles. Classical biological control of ACP has been applied in Florida with the release of the tiny wasp parasitoid *Tamarixia radiata*. This parasitoid became established, but with little affect on ACP. Introduced predators and parasitoids have suppressed several long-established hemipteran species (aphids, whiteflies, and scale insects) over decades in Florida. However, to reduce *C*Las transmission (i.e. acquisition and inoculation) requires more severe reductions in ACP populations than has been seen for other hemipterans. The only report of successful HLB mitigation through biological control was accomplished in Reunion Island, a small, non-continental landmass, unlike Florida. Thus, biological control alone is not likely to reduce ACP populations sufficiently to provide HLB mitigation.

To date, significant reductions in psyllid populations have been achieved in Florida only through the application of insecticides. Recommendations for ACP suppression, based on research, are published in an annual University of Florida management guide. This guide influences grower management practices. However, growers often experiment as well, leading to variable practices in orchards. Presumably, implementation of *Recommendation O-1* would result in more uniform practices.

The principles of integrated pest management (IPM) are becoming and should become more influential. There are continuing efforts to improve the monitoring of ACP populations, by employing new insect traps, sentinel plants, and strategies for monitoring pest movement through mark and recapture experiments. Generally, production managers are aware of the increasingly important and potentially adverse consequences of inappropriate application protocols and frequent insecticide sprays, including farm worker exposure, the development of resistance to insecticides, reduction in numbers of beneficial insects, and contamination of groundwater. Additionally, the economics of heavy reliance on insecticides must be carefully examined and compared with other methods of control. These comparisons should address not only production costs, but also other costs such as potential harm to the environment (see *Recommendation O-4*).

Psyllid females strongly prefer to lay eggs in the new flushes of growth that are produced by all citrus, but occur on young trees throughout most of the year. Insecticide applications can be timed to protect flushes, whether they occur as the result of the natural growth cycle of citrus or because of tree hedging operations. Young trees require aggressive ACP suppression involving the soil-applied systemic insecticides aldicarb or imidacloprid. Other recommendations take into account the relatively low psyllid populations that occur during winter, when broad-spectrum foliar sprays may be applied to mature trees to suppress populations of overwintering adults.

Production managers and researchers are actively testing modifications to current approaches. Low-volume spraying is gaining widespread use; it allows application of products to large areas in a short time, which is a central component of area wide management approaches to HLB mitigation (*Recommendation O-1*). New active ingredients and adjuvants are being examined for their ability to provide more effective and more environmentally friendly ACP suppression.

The reservoir of scientific knowledge of psyllids has expanded since the discovery of ACP in Florida in 1998. However, additional knowledge about the behavior of and chemical communication by ACP, infected with *C*Las or not, has potential for revealing points at which the disease cycle might be interrupted (*Recommendation NI-7 and L-3*). Data support the existence of female sex pheromone(s) and plant volatile attractants. Several observations suggest that specific chemical compounds can attract or repel ACP and could be used in strategies which direct ACP to congregate at sites where their destruction could be more easily accomplished. Knowledge of the ACP and CLas genomes (*Recommendations N-11* and *NI-6)* may contribute significantly to acquiring and interpreting the needed new information. Information on *C*Las effects on ACP and *C*Las transmission rates underlies predictions on HLB spread and other HLB investigations. However, this information is very limited, and current estimates of transmission rates vary widely.

Methods that potentially could be applied to ACP suppression include the sterile insect technique (SIT) and related genetic technology that would involve release of reproductively debilitated ACP males. However, the dense populations achieved by ACP, the serial mating behavior of ACP females, and the lack of methods for mass rearing of ACP all speak against the possibility of HLB mitigation by altered ACP release. Therefore, *Recommendation L-4* advocates limited or no investment in SIT and ACP genetic technology at this time.

Genetic Transformation of Mature Citrus Tissue

Many protocols for citrus transformation have been published, most initiated from juvenile citrus tissue. For a practical citrus-transformation program with a reasonable time to creation of productive stock, methods for the transformation of mature citrus tissue, or an equivalent process using altered juvenile tissue, will probably be required (*Recommendation L-1*).

Once a system for citrus transformation is developed, several transgenic mechanisms for citrus resistance become possible. Expression of anti-*C*las agents is one mechanism. A potential source of anti-*C*las agent is the various anti-bacterial peptides produced by both microbial and higher organisms. Many of these peptides are well characterized as to their target and specificity. Various proteins and peptides with specific insecticidal activity could be produced in citrus from transgenes. Systemically acquired resistance (SAR), which is the activation of a systemic defense reaction in a plant, is also currently being exploited by both chemical and transgenic approaches. Another approach has ACP as the target and applies the phenomenon of RNA silencing (see

Recommendation NI-9) activated by double-stranded RNAs that would be phloem-delivered in the transgenic plant to adversely affect feeding insects.

A Plant Virus-Derived System for Gene Expression in Citrus

The *Citrus tristeza virus* (CTV), itself a significant pathogen under certain but avoidable circumstances in Florida, has been modified by the laboratory of W.O. Dawson of the University of Florida to create a virus gene-expression vector. Unlike almost all other virus-based vector systems for gene expression in plants, some of the CTV vector constructions were found to maintain gene expression for more than 6 years rather than for days or weeks. The usual application for a transient expression vector such as CTV is as a test bed for evaluating gene constructions intended for later use as transgenes. However, the longevity of expression shown by CTV constructions could allow direct application of this technology to confer commercially useful resistance to *C*Las and to do so in significantly less time than would be required for creation and deployment of a resistance gene in genetically transformed citrus.

Whether new genes are introduced by citrus transformation or by application of a CTV vector, there will be requirements for field testing and to satisfy regulatory requirements. There will also be intellectual property considerations derived from the gene constructions or transformation methods used. These are time-consuming and costly steps that must be taken after the particular approach and construction have passed the proof-of-concept stage. However, there is another important hurdle: public acceptance, which will require public education.

Prophylactics and Therapeutics

A few current research projects aimed at mitigating HLB use model systems that do not include citrus or *C*Las or ACP in experiments, or may include only one of them. None of the three, citrus, *C*Las or ACP, is an easily tractable experimental subject. As the text for *Recommendation NI-5* describes, high throughput assays have been or can be developed that use multi-well plates to contain infected or uninfected seedlings or plant stem sections, individual insects, or individual bacterial cultures. Genome sequence information (*Recommendations NI-4*, *NI-6* and *NI-11*) may guide the selection of chemical libraries to be screened in the multi-well plates in a quest for new bactericides or other therapeutic agents, or prophylactic agents, for use in HLB mitigation (*Recommendation L-2*) or for the discovery of ACP repellents. The screening and testing of such agents would be materially assisted if *in vitro* culture techniques for *C*Las were available (*Recommendation NI-10*). A model system could be the most direct route to an anti-*C*Las spray that could protect existing plantings, i.e., a so-called "silver bullet."

CONCLUSION

HLB is the latest, and most serious, challenge that nature has presented to the Florida citrus industry. A successful response will require acceptance of the urgency of the situation and willingness to cooperate and persist in managing the disease in the near- and mid-term while new solutions are being developed and implemented. The HLB problem requires an unprecedented degree of cooperation among producers, processors, government officials, and scientists, and of course among their respective institutions. However deficient is our current arsenal for fighting HLB, the potential for progress against this disease remains distinctly hopeful.

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Introduction

The devastating symptoms of a disease of citrus had been recorded in the late 19th century in China. These symptoms were usually attributed to nutritional problems. The disease was further reported in various parts of Asia and Africa. In the 1950s, the Chinese researcher K. H. Lin showed the disease to be the result of a transmissible agent. However, all of these observations and research results were virtually unnoticed by researchers in the West at the time of their publication. The disease is commonly referred to in the United States as "citrus greening". Citrus greening is also known as huanglongbing or HLB; huanglongbing is translated from Chinese as "yellow shoot disease". HLB became generally recognized as a threat to the citrus industry in the 1960s and 1970s, the period in which a bacterial etiology for the disease was suggested from electron microscopy studies and its transmission by a small insect, any of several species of psyllids, was documented. One of these psyllids, the Asian citrus psyllid (ACP), was discovered in Florida in 1998, and in 2005 symptoms of HLB were observed by scouts in urban plantings in southeastern Florida. Both the ACP and HLB rapidly spread into the commercial citrus growing areas of Florida.

Florida citrus is recognized as an industry that has been assaulted by hurricanes and freezes, numerous diseases and pests, shortages of water, and urban encroachment. In this panoply of threats, citrus greening is recognized as the greatest threat, a threat to the very survival of the industry.

BACKGROUND

In early 2008, the Florida Department of Citrus (FDOC) requested the assistance of the National Academy of Sciences (NAS) in addressing the citrus greening problem in Florida. The NAS was asked to convene a panel of experts, with the charge of identifying research priorities for citrus greening (HLB) and creating a request for research proposals (RFP) based on the identified research priorities. The NAS was also requested to review and evaluate, for scientific and technical merit and feasibility, the proposals that would be submitted in response to the RFP. The panel of experts met in April of 2008 and identified research priorities. The National

Research Council (NRC) of the NAS developed a list of eight subject areas under which research proposals on HLB could be considered and identified potential review panel members for each. The review panels were appointed, and during the fall of 2008 the review panels met in Washington, D.C., each panel evaluating the corresponding set of proposals. Subsequently, a new panel composed of the chairs of the individual panels and the chair of the new panel, who had not participated in the initial process, evaluated all of the proposals. In December of 2008, the new panel submitted a list of research proposals that were deemed worthy of funding for consideration by the FDOC, which had allocated $20 million for research on citrus HLB and other serious citrus diseases.

PURPOSE AND SCOPE OF THE STUDY AND THE FORMATION OF THE COMMITTEE

As part of its request for assistance with the citrus HLB problem, the FDOC also asked the NAS to assemble and support a study committee that was assigned the task of developing a strategic plan for dealing with the citrus HLB problem. The strategic plan is to deal with the near-term mitigation and research efforts and, in the longer term, organizational and technical approaches to reducing the damaging effects of this devastating disease. The formal proposal to develop the strategic plan was approved by the Executive Committee of the NRC's Governing Board on March 12, 2008. However, the Statement of Task was subsequently revised to include recommendations for ways that the Florida citrus industry can improve its capacity to respond to citrus HLB in a more comprehensive way. The revised Statement of Task, as approved in March, 2009, may be found as Appendix A of this report. In April of 2009, the NRC formed the Committee on Strategic Planning for the Florida Citrus Industry: Addressing Citrus Greening Disease (Huanglongbing). Biographical information on the committee members is presented in Appendix B. Given the urgency of the citrus HLB situation in Florida, the study was placed on a fast-track schedule for completion in early 2010 rather than allowing the usual 18 months or more for completion of a NRC committee report.

CHARGE TO THE COMMITTEE

The Committee was asked to examine the following:

- The current citrus disease situation in Florida and the status of public and private efforts to address citrus greening and other diseases; including lessons learned
- The capacity of the industry to mobilize a scientifically-based response to current disease threats and to translate scientific advances into products and services for the protection of Florida citrus industry in the short and long term
- The relationship of the industry to public, academic, and private research, and to regulatory and funding organizations at the state and federal level, with respect to controlling citrus greening and developing a comprehensive solution to citrus greening and other diseases.

COMMITTEE'S APPROACH TO THE STUDY

Part of the challenge to producing this report was the relatively short period given to the committee to complete its task. This was due to the urgency of finding ways to manage citrus greening before it becomes economically infeasible to produce citrus in Florida. The study began with a consultation with representatives from the Florida citrus industry, representatives from federal and state agencies, and the university research community, most of whom formed the study's liaison committee (Appendix C). The purpose was to get first-hand information on 1) the impacts of citrus greening and the actions taken by citrus growers and 2) the ongoing regulatory and research activities to address citrus HLB.

As part of the information-gathering phase, the committee also asked experts from different scientific and technological fields to submit a short written report on a topic selected by the committee. Based on the submitted reports, the committee selected a subset from the group to meet with the committee in person or by telephone to discuss new research findings, promising results, and technologies with potential application to the mitigation of HLB. Presentations were given at the open session of the second Committee meeting in Washington, D.C. The committee met a total of three times, once in Florida (May, 2009) and twice in Washington, D.C. (July and September, 2009). Except for testimony collected from the selected experts, the meetings were in closed session. A list of all oral presentations and speakers and sources of written statements are given in Appendix D.

All of the members of the review panels, the strategic planning committee, and the liaison committee were selected by the NRC. As indicated above, other experts who provided input were selected by the committee. None of the people who served on the panels or committees or provided testimony were remunerated for their service.

ORGANIZATION OF THE REPORT

This report, the result of a fast-track study, is composed of 5 chapters and 13 appendixes. Chapter 2 provides a historical review of the citrus industry, citrus diseases and other constraints on citrus production, and details on HLB, including the discovery of its infectious nature, likely causal agent and insect vector and current methods for mitigating the disease. Some gaps in research efforts are also indicated. Also examined in Chapter 2 are the roles of, and relationships between, federal, state, university, and industry organizations with connections to citrus production and processing.The focus of Chapter 3 is on research results related to HLB and their possible applications in HLB mitigation. Prior to the invasion of HLB into Florida, published research results on the bacterium and insect vector were few. Subsequently, there has been a dramatic increase in publications on these topics. Chapter 4 presents the specific recommendations of the Committee as they relate to organizational changes, informational initiatives, and research and technology projects with near-term, near- to intermediate-term, and long-term potential. An analysis of the factors supporting and not supporting the recommendation is presented with each recommendation. Chapter 5 contains the Committee's recommendations for implementation of recommendations and a comparison of various models for supporting and managing research. Preparing for the future of the Florida citrus industry is also a topic of Chapter 5.

2

Challenges to Citrus Production

ECONOMIC IMPORTANCE OF CITRUS: WORLDWIDE, UNITED STATES, FLORIDA

Commercial citrus fruit are produced in about 140 countries. The primary producers are Brazil, the Mediterranean Basin, the United States, and China. Citrus fruit ranks first internationally in trade value among all fruits (Norberg, 2008; UNCTAD, 2009). Sweet oranges are the major fruit grown and represent approximately 70 percent of the citrus output. According to the Food and Agriculture Organization of the United Nations (FAOSTAT, 2007), four countries account for about 60 percent of the worldwide orange production: Brazil (32 percent), United States (14 percent), India (6 percent), and Mexico (7 percent) (Figure 2-1).

Four states, Florida (68.7 percent), California (27.5 percent), Texas (2.7 percent), and Arizona (1.1 percent), produce virtually the entire US commercial citrus crop. Sweet oranges dominate Florida's citrus production (83.6 percent) followed by grapefruit (12.6 percent) and specialty fruit consisting of mostly tangerines and tangelos. Florida produces very few lemons.

Two areas dominate the production of oranges for juice: São Paulo State, Brazil (50 percent) and Florida (35 percent) (Norberg, 2008). More than 95 percent of Florida's orange production is processed into juice, accounting for essentially all of the US orange juice production. China is another major orange juice producer. Brazil exports about 90 percent of its juice production including a considerable amount to the United States, whereas only 10 percent of Florida's production is exported internationally.

Important producers of sweet oranges for fresh consumption are Spain, Turkey, South Africa, and California (United States). Fresh market oranges bring much higher returns per acre than juice processing oranges. Mandarin production is concentrated in China, Japan, and other Asian countries, but high quality export fruit is produced in South Africa, Spain, and other countries of the Mediterranean Basin. Lemons are produced in Argentina, California, Italy, Spain, and other Mediterranean countries.

Florida grapefruit is grown predominately for fresh fruit but a considerable portion of the crop is processed for grapefruit juice as well. Florida is the largest US producer, commanding about two-thirds of the total. Florida produces about 30 percent of the world's grapefruit with Mexico, Cuba, and Turkey contributing significantly to world production. Japan is the largest importer of fresh Florida grapefruit and Canada is the leading importer of Florida fresh oranges and specialty citrus fruit (USDA-APHIS, 2006; USDA-NASS, 2008, 2009).

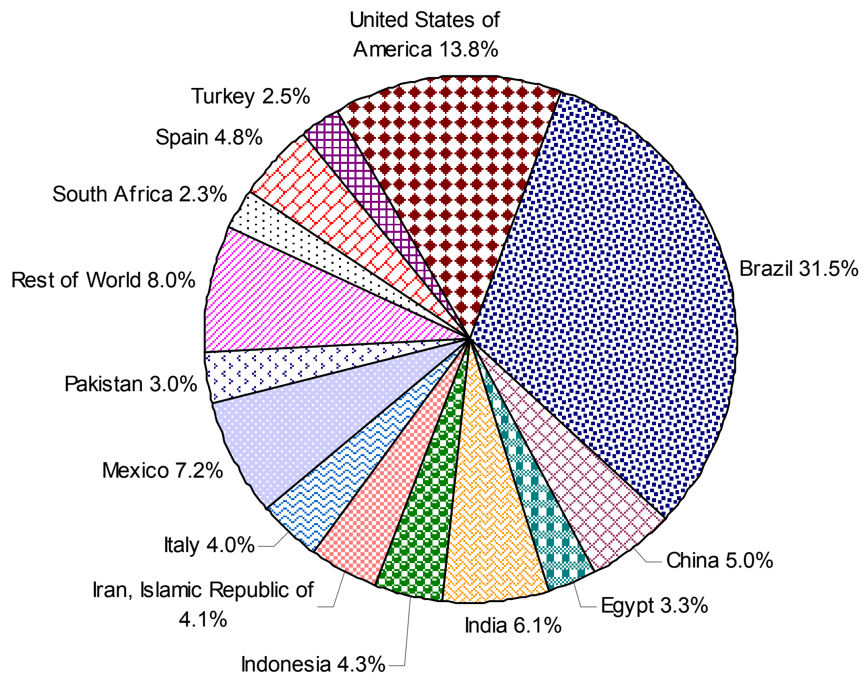

FIGURE 2-1 Worldwide orange production, percent total production, 2005–2007.
Source: FAOSTAT, 2007.

The Florida citrus industry is estimated to have a $9.3 billion economic impact for the state. Approximately 80,000 full-time equivalent jobs (grove employees, seasonal pickers, haulers, processors, packers, and managers) are involved, earning a combined annual wage of $2.7 billion or about 1.5 percent of the state's wage income. Most of the fruit is harvested by hand although harvesting machines have been developed and are used for harvest of about 5 percent of the processing fruit. Nearly all of the hand harvesters are transient labor, mostly from Mexico or Haiti. Citrus is a major segment of Florida's agricultural industry, accounting for an estimated 21.1 percent of cash farm receipts in 2005 (Norberg, 2008).

HISTORY AND EVOLUTION OF THE CITRUS INDUSTRY IN FLORIDA

Cultivation of citrus is believed to have begun in Southeast Asia roughly 4,000 years ago. Trade and cultivation moved slowly west to Northern Africa, the Mediterranean, and then to southern Europe by the Middle Ages. Christopher Columbus is thought to have brought the first citrus seeds on his second voyage to the New World in 1493. The Spanish explorer, Ponce de Leon, is credited with planting the first orange trees near St. Augustine, Florida sometime between 1513 and 1565. French Count Odet Philippe first introduced grapefruit to Florida in 1806. He later planted the first grapefruit grove near Tampa in 1823 (Webber et al., 1967; Florida Citrus Mutual, 2009; UNCTAD, 2009).

Commercial farming of citrus in Florida began in the mid-1800s, motivated by favorable growing conditions in Florida and a growing demand for the attractive, healthful fruit. The fledgling industry's growth was facilitated by improved commercial rail transportation systems along the east coast that opened the market for the fruit to northeastern United States. By the end of the Civil War, annual citrus production in Florida reached roughly one million boxes. By 1893, production was about 5 million boxes.

Florida's hot and humid climate has made the success of its citrus industry possible, but periodic winter freezes and hurricanes have shaped the industry's development. The Great Freeze of 1894–1895 proved devastating. Orchards throughout the state were ruined and most production on the Gulf Coast of other states was discontinued. A trend to move groves further south in Florida followed this freeze. This gradual movement south has continued following subsequent severe freezes mainly in the 1930s, 1960s, and 1980s, resulting in larger acreages in south Florida and disappearance of most citrus from the more northerly counties. Hurricanes have had an immediate negative effect on orange production. From August 2004 to October 2005, Florida was hit by four significant hurricanes (Figure 2-2). Based on figures from the US Department of Agriculture- National Agricultural Statistical Service (USDA-NASS) (2005), the 2003–2004 season saw a near record orange crop of about 240 million boxes of fruit. But, following the string of hurricanes, the figure for the 2004–2005 season was down to about 150 million boxes. For oranges alone, the decline in yield after the hurricanes was 38 percent (Table 2-1).

In contrast to California fruit, Florida oranges have a thinner skin, are more difficult to peel, are more subject to transit and handling damage, and are less attractive. These factors become irrelevant when the fruits are converted to juice. Processing into juice takes advantage of the characteristic "juiciness" of Florida fruit brought about by the state's hot, humid climate. The earliest harvest of fresh market oranges begins in October, for Hamlin, Parson Brown, Ambersweet, and Navel varieties with some cosmetic packinghouse eliminations being processed for juice. The bulk of the early orange processing begins in December. Early and mid-season varieties are harvested throughout the winter and harvest of late Valencia oranges begins in early April and continues into June.

Following World War II, commercially feasible methods to process and freeze fresh fruit into concentrated juice were invented. This was a major boon to the industry, especially in Florida. Frozen juice has an extended shelf life that permits the storage of inventory to smooth out variable seasonal supply due to adverse growing conditions caused by droughts and hurricanes. The use of frozen concentrate permits blending of juice stock to provide consumers with a consistent high quality product. The convenience of a juice form also increases consumption over what it would be if only fresh fruit were available. More recently, shipping and handling methods have improved and fresh juice can be transported without producing frozen concentrate and "not from concentrate" products have become a popular product in the United States (Brown, 1995).

Brazil, specifically São Paulo State, is Florida's key competitor in the juice market. The Brazilian coffee crisis of the 1930s led to the shift toward citrus production. The crisis was caused by a huge oversupply of coffee beans brought about by government price support programs that actually contributed to further cultivation of trees thereby exacerbating the oversupply. The worldwide Great Depression sharply reduced demand for coffee in the 1930s. Brazil entered into a period of political and economic instability that, in combination with

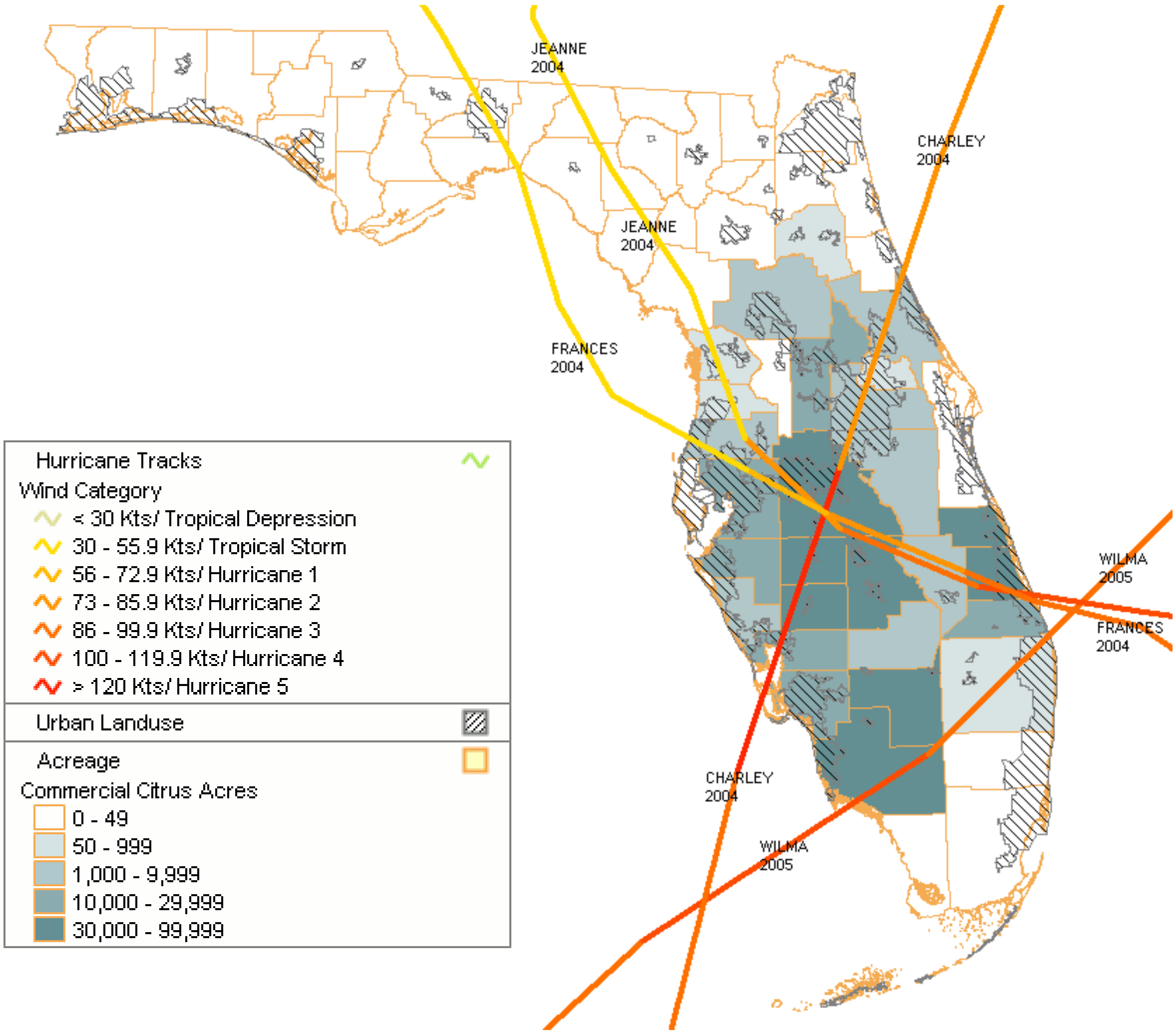

FIGURE 2-2 Path of hurricanes Charley (August 13, 2004), Frances (September 5, 2004), Jeanne (September 26, 2004), and Wilma (October 24, 2005).
Source: NOAA-CSC, 2009.

Table 2-1 Impact of 2004–2005 Hurricanes on Florida Citrus Crop Yield (Number of Boxes)

Fruit	2003–2004	2004–2005	Percent Decline
Orange	242,000,000	149,600,000	38
Grapefruit	40,900,000	12,800,000	69
Specialty	8,900,000	7,000,000	19

Source: USDA-NASS, 2005.

reduced demand, led to a collapse in coffee prices paid to growers. Growers shifted to citrus and this began a period of growth for this crop. Growth in the 1960s was particularly steep as Brazil ramped up production in response to freezes in Florida that destroyed orchards and seriously affected supply. Brazil set out to be a source of alternate supply to fill the existing demand for citrus products in the United States and Europe. By the 1980s, Brazil had become the largest supplier of citrus fruit and juice.

Urbanization and diseases have been critical factors in the recent history of the Florida citrus industry. According to the 2000 US census, Florida ranks seventh in the US in population growth (23.5 percent) from 1990–2000, almost double the US average of 13 percent, and corresponds to a daily population growth in Florida of 830 (US Census Bureau, 2009). The housing boom over the past few decades, now a burst bubble, led to rapid residential development in the state especially near the coast. Developers purchased grove land at premium prices, by agricultural standards, in part because acreage was plentiful and still relatively inexpensive for real estate. Developed properties are taxed at a much higher rate than agricultural lands, so public officials are disinclined to oppose the conversion of groves to residential and commercial development. Urban encroachment means that many citrus-growing areas are no longer isolated. Current levels of production and citrus acreage are sharply lower than the levels of the mid-1990s through 2004 (Figure 2-3 and 2-4). The number of juice processors has also declined sharply. There were 37 processors in Florida in 2001, but only 15 remained in 2008 (Norberg, 2009). The agricultural alternatives for most citrus land are cattle pasture or pine forests, neither of which is highly profitable, so growers have few options.

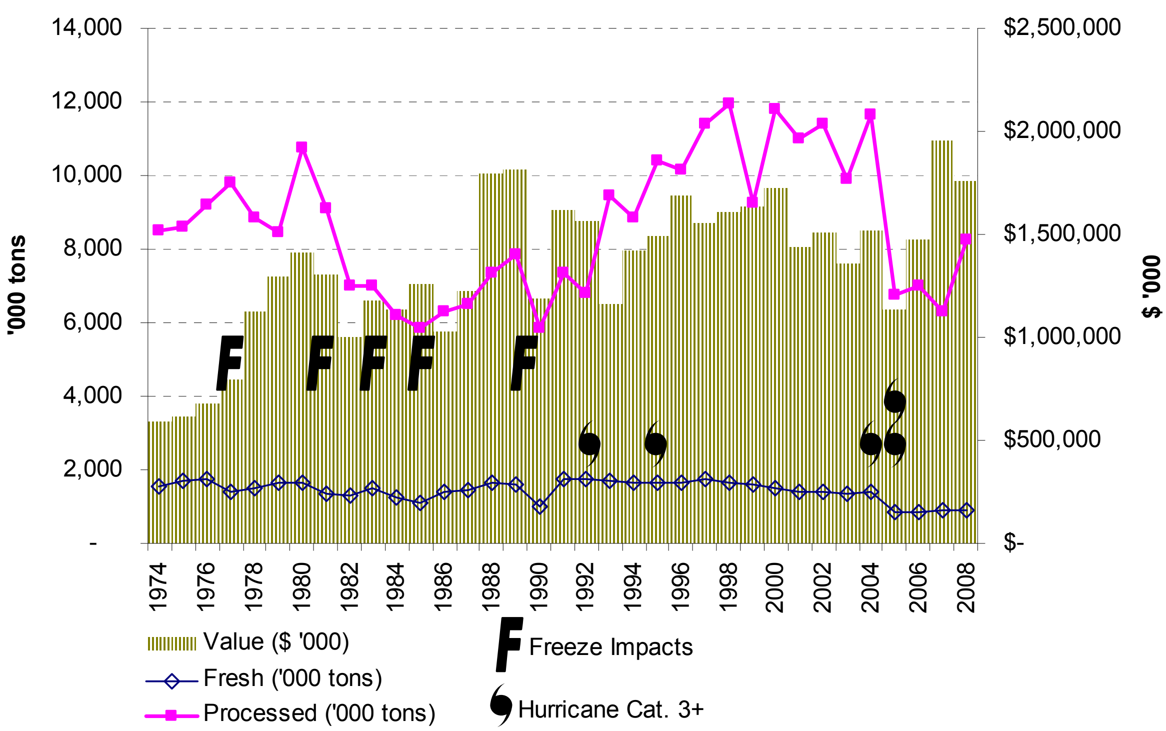

FIGURE 2-3 Florida citrus production in tons and dollar value.
Source: USDA-NASS, 2008.

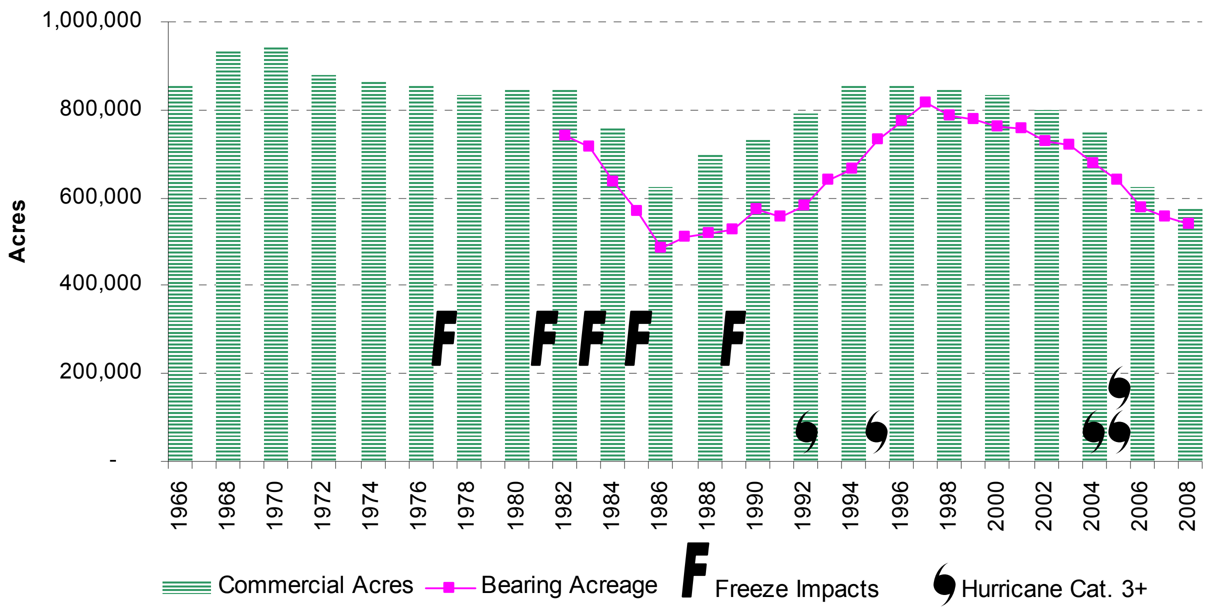

FIGURE 2-4 Acreage of existing commercial and bearing citrus groves.
Source: USDA-NASS, 2009.

Plant diseases such as citrus canker have had a negative effect on citrus production and economics. The eradication program for citrus canker was responsible for the loss of 70,000 acres of groves, and the regulations on the movement of fruit from areas with the disease have greatly affected the ability of the state to market fresh fruit. Over the last 20 years, *Citrus tristeza virus* (CTV) caused the decline and death of most trees on sour orange rootstock, which originally constituted 25–30 percent of the acreage in Florida, but most of that acreage was replanted gradually as trees were lost.

The greatest immediate threat to both the processing and fresh fruit industries in Florida is the recently arrived disease known as citrus greening or huanglongbing (HLB). Developing measures for combating HLB is the principal topic of this report. The insect vector of the HLB-associated bacterium in the Americas is the Asian citrus psyllid (ACP), which was reported in Brazil in 1942, but was not reported in North America until it was found in 1998 in Florida. Although it cannot be known whether small, unetected populations of ACP predated discovery, it is clear that ACP was widely distributed by 2000, having been reported in 31 counties in the state, so that eradication was no longer conceivable. The ACP reportedly arrived in Texas in 2001 on potted *Murraya* plants that originated from Florida (Mead, 2009). HLB itself was discovered in Brazil in 2004 and in the Miami area of Florida in August of 2005. The origin of HLB in Florida is unknown but may have been from budwood imported from Asia. The exclusion of pests such as ACP and the HLB bacterium clearly would have been the most effective control measure. Improving current measures for excluding pests and pathogens would likely benefit Florida citrus, but pest exclusion is a topic beyond the scope of this report. HLB is now present in all 34 Florida counties that have commercial citrus fruit production (FDACS-DPI, 2009), but is most prevalent in the southern areas of the state (Figure 2-5). The spread of HLB in

Florida is considered to have occurred too rapidly to be accounted for by ACP flights, and it is likely that long-distance spread of ACP occurred due to commerce or hobbyist activities involving backyard and ornamental citrus. HLB is widespread in Brazil and many other citrus-producing regions. HLB represents a serious threat to the citrus industry worldwide.

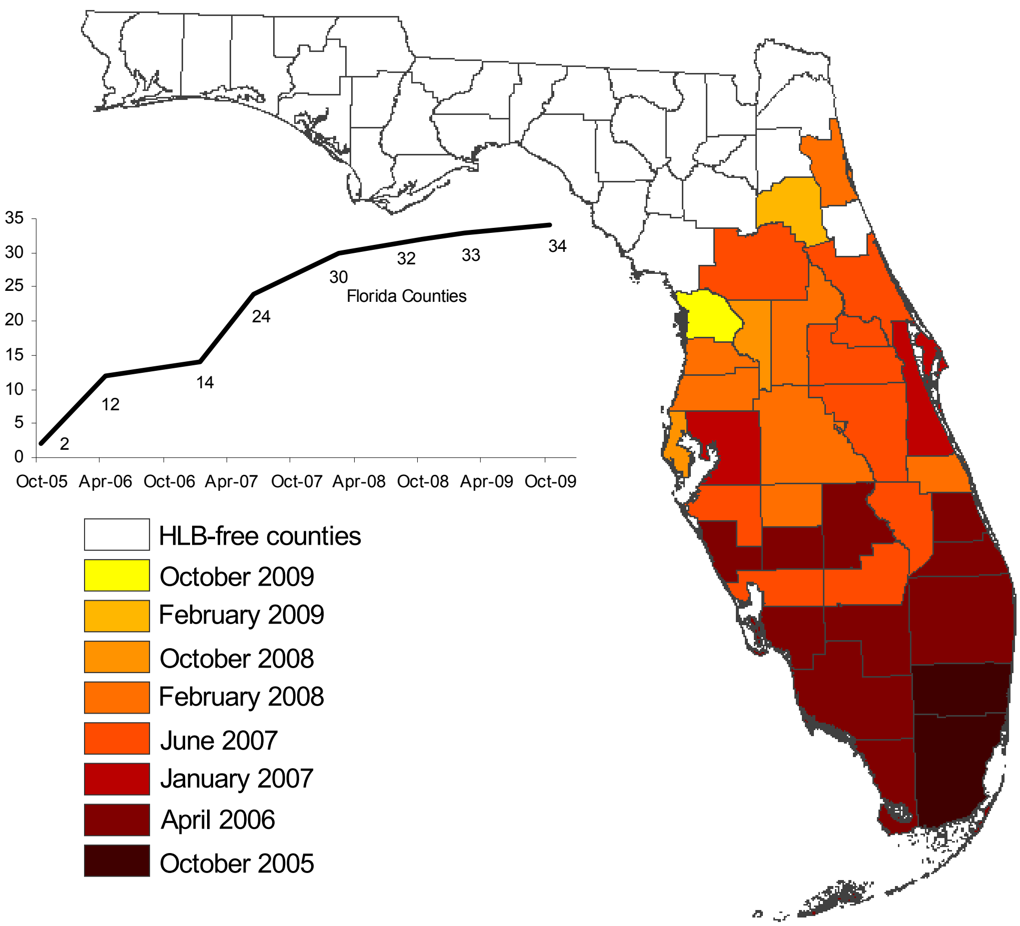

FIGURE 2-5 Distribution of HLB in Florida from October 2005 to October 2009.
Source: FDACS-DPI, 2009.

ECOLOGY, CLIMATE, WATER AND FLORIDA CITRUS

Landscape Factors that Contribute to Pest Outbreaks in Florida

It has been estimated that approximately 4,500 arthropod species have been introduced into the United States and of this total, approximately 1,000 insect and mites have become crop pests (Pimentel et al., 2000). Because of Florida's geographic position in the United States, it is subject to intense pressure from invasive species. Florida's 1,350 miles of coastline, second only to Alaska's, provides easy access for pests that arrive by aerial movement or with humans from tropical areas in the Caribbean, Central and South America where arthropods may reproduce continuously without a winter break. Florida's population of 18.3 million people places it 4th among US states and with about 6 percent of the US total. This population receives shipments of agricultural produce from around the world through the state's large airports and seaports. Despite surveillance by the US Department of Agriculture- Animal and Plant Health Inspection Service (USDA-APHIS), these ports remain major entry points for new pests that threaten Florida's agriculture. Once in Florida, pests can be transported intra- and inter-state by movement of propagative materials, foliage or soil and become established.

The 600,000 acres of commercial citrus in Florida currently is confined to the southern two-thirds of the peninsula exclusive of the urban Miami area and the Everglades (Figure 2-6). The highest point in Florida is only 345 ft in elevation. The flatness of Florida's landscape facilitates planting, harvest, and transport of agricultural produce but also results in a lack of natural barriers to the movement of ACP and HLB, e.g., in storms. In the Central Ridge area, citrus is planted on deep sandy soils. Citrus in southwestern and coastal areas is planted on "flatwoods" which also have sandy soils, but are often very shallow with rooting depths of no more than 1 meter and contain some organic matter. In such areas, citrus is planted on raised, two-row beds with ditching to avoid flooding in the summer rainy season. Displacement of citrus from urbanized coastal areas has resulted in a larger proportion of the crop being at risk from frost damage.

Urban encroachment on production areas means that even if commercial citrus producers use effective management practices to control ACP in their groves, nearby residential plantings may serve as a reservoir for ACP and HLB. Abandoned orchards and those that receive minimal care serve as major sources of pests and diseases that may infest commercial orchards or nursery stock. The slowing of home building recently has resulted in groves purchased for housing being left undeveloped and becoming potential sources of ACP and HLB.

Climatic Conditions that Contribute to Pest Outbreaks in Florida

Generally speaking, Florida's sub-tropical climate and year round vegetation in and around citrus groves allows a larger number of generations of insects to be produced and leads to more frequent application of management practices, such as insecticides, than would be found in temperate areas. This situation has potential negative consequences for farm workers and the general environment. Accordingly, Florida also has cases of insect resistance to insecticides (Omoto et al., 1995). Although resistance may originate in one particular location due to the local management practices, such resistant insects may be able to move long distances. In Florida, such long-distance movement occurs regularly because of hurricanes and summer thunderstorms. From 1851 to 2008, there were 96 category-3 to 5 hurricanes that made landfall on the eastern US coast (Texas to Maine). Thirty nine percent of these landfall events occurred in Florida (AOML, 2009).

Water Use, Groundwater Protection, and Citrus Production

Water, once a cheap and plentiful resource in Florida, is becoming a precious and valuable commodity for all sectors of Florida's economy, including agriculture and tourism. Florida relies on groundwater pumped from permeable aquifers underground to supply drinking water to more

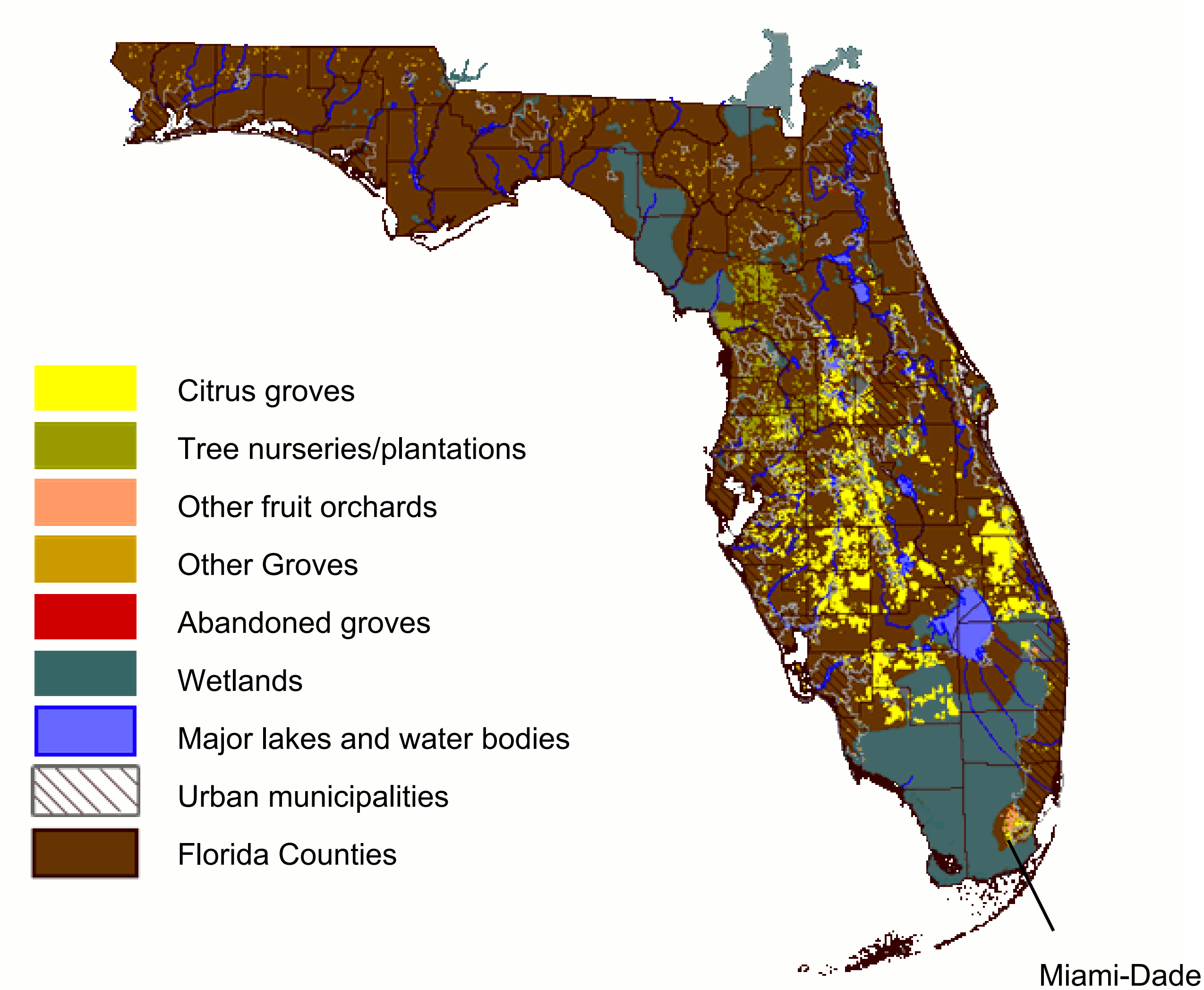

FIGURE 2-6 Citrus production areas in Florida.
Source: FGDL, 2007.

than 90 percent of its population (FDEP, 2009). Water consumption continues to rise and the water levels in the aquifers continue to drop, sometimes leading to geological disturbances such as sinkholes and saltwater intrusion into city wells (Hutson et al., 2004; Barnett, 2007). Much of this increased consumption is due to urban activities such as the irrigation of lawns and golf courses associated with the fast-growing housing developments throughout the state. However, farms are also large water consumers, using nearly half of Florida's public supply (Barnett, 2007). Citrus is a tropical/subtropical crop; therefore it requires substantial irrigation when grown in drier climes. Unlike other evergreen fruit trees such as avocado and mango, citrus trees continuously replace their leaves as they grow. Further, citrus trees have a relatively shallow root system as compared to deciduous fruit trees such as apple and walnut. In Florida, depending on soil type, root depths range from 18 inches in the coastal flatwoods to 11 feet in central Florida (Boman and Parsons, 1999). Studies in Australia have shown that mature citrus may require 7–8 mega liters (ML) of water per hectare while young citrus plantings may require 2–5 ML per hectare annually (Falivene et al., 2003). Reducing irrigation by 20 percent or more resulted in significant citrus crop losses. Citrus water requirements may be less in Florida than in Australia due to Florida's lower temperatures and higher humidity, but the requirements nevertheless are substantial.

Despite relatively high rainfall, virtually all Florida commercial citrus has microsprinkler irrigation systems installed to meet the crop water needs in the state's soils, which are up to 95 percent sand (Reddy et al., 1992). Microsprinklers consume less water that the older overhead irrigation systems they replaced. Drip irrigation is not effective in Florida's very sandy soils, except for young trees (Parsons and Morgan, 2004). Rainfall in Florida is confined largely to the summer season and irrigation is most needed in the dry spring. In the summer months, citrus grown in "flatwoods" groves has the opposite problem and requires good drainage to avoid flooding.

The Florida Department of Environmental Protection (FDEP) has the primary role of regulating public water systems and its responsibility for ground water is divided into two individual programs—ground water protection and ground water regulatory (FDEP, 2009). Citrus production is impacted by both programs. The FDEP requires permits to pump ground water and controls the volumes that can be pumped at any time. The water is controlled by five management districts that may impose restrictions as needed on residential, industrial, or agricultural use. For groundwater protection, the FDEP has the responsibility for monitoring fertilizer and pesticide levels in the water to make sure that they do not exceed the Environmental Protection Agency (EPA) standards. Use of agricultural products, especially pesticides, in sandy soils is particularly contentious, and is likely to become even more so now that ACP and HLB are threats to the citrus industry. Some of the management practices in place are of concern for water quality. As is stated in the University of Florida 2010 Florida Citrus Pest Management Guide, "the only soil-applied insecticide that has been shown to provide any reduction in psyllid numbers on large trees is aldicarb" (Rogers and Dewdney, 2010). Studies conducted in the 1980s in the central sands area of Wisconsin demonstrated that aldicarb can be found in wells (Rothschild et al., 1982) and follow-up studies suggested a health risk from consumption of aldicarb-contaminated groundwater (Rothschild et al., 1982). Under Florida conditions, aldicarb degrades relatively rapidly, but nevertheless has been found in some shallow water wells (Jones and Back, 1984; Forrest and Chris, 1986). Thus, aldicarb is limited to applications only in the dry season (November–April) and cannot be applied within 1000 ft of a drinking water well. Although aldicarb use under the indicated conditions is regarded as safe, it is desirable to rely on other methods and other insecticides for long-term control of ACP and HLB, to minimize risks to human health and the general environment.

Other Environmental and Landscape Management Issues

Commercial citrus production is close to being a monoculture crop. However, herbicides are applied only under the trees, leaving grasses and other plants in row middles and ditches with considerable vegetation surrounding most blocks in flatwoods groves. Crop monocultures facilitate mechanization of production and harvesting activities and allow specialization in marketing. However, monocultures generally provide less biological diversity and thus tend to be less ecologically stable than polycultures. Generalist natural enemies, primarily predators, are usually less abundant in monocultures since they have fewer alternative hosts to sustain them before the pests on which they can feed build up to damaging levels on the cash crop (Landis et al., 2000; Bianchi et al., 2006). Thoughtful habitat management and introduction of plant diversity into a cropping system can help reduce pest populations and their damage on the cash crop by providing natural pest enemies with resources such as nectar, pollen, physical refugia and alternative hosts. There are many examples of "ecological engineering" tactics in annual crops and a few in perennial crops as well (Gurr et al., 2004). Three examples are summarized below, and described in more detail in Chapter 3..

Growers and researchers in Vietnam have noted that interplanting citrus with guava almost entirely negated infestations by citrus psyllids and, as a consequence, the citrus trees remained free of HLB (Stover et al., 2008; L. Stelinski citing unpublished report from Vietnam). Florida scientists are exploring the use of guava and the underlying biochemistry of guava's putative capacity to limit or prevent infestations of citrus psyllids.

In New Zealand, buckwheat ground cover has been used to enhance the parasitism of a leafroller insect, a major pest of grapes. Apart from enhanced biological control, mixed plantings of crops may disrupt an insect's ability to locate or establish on a plant species (Price, 1984).

Trap cropping is the use of plant stands that are, per se or via manipulation, deployed to attract, distract, intercept, retain, and/or reduce targeted insects or the pathogens they transmit in order to reduce damage to the main crop (Shelton and Badenes-Perez, 2006). The trap crop may be treated to suppress the "trapped" insect population.

DISEASES AND PESTS THAT THREATEN CITRUS PRODUCTION

HLB (Citrus Greening): Impact, History, and Disease Spread

Impact of HLB

HLB is a destructive disease, and probably is the most serious disease of sweet orange, mandarin and grapefruit trees. It is destructive irrespective of rootstocks or whether the trees are grafted or are seedling trees. The yield of affected trees is not only reduced considerably by continuous fruit drop, dieback, and tree stunting, but also by the poor quality of fruits that remain on the trees.

HLB epidemics take several years to reach high incidence levels. The temporal progress of HLB incidence is dependent on (i) vector populations, (ii) extent of the inoculum reservoir, and (iii) age of the grove at first infection. The disease progress in the orchard can be relatively fast, reaching more than 95 percent incidence in 3 to 13 years after onset of the first symptoms (Catling and Atkinson, 1974; Aubert et al., 1984; Gottwald et al., 1991; Gatineau et al., 2006; Gottwald et al., 2007a; Gottwald et al., 2009). Severe symptoms have been observed 1 to 5 years after onset of the first symptoms, depending on the age of the tree at infection time and on the multiplicity of infection (Lin, 1963; Schwarz et al., 1973; Aubert, 1992). As the disease severity increases, the yield is reduced and makes the orchard production uneconomical in 7 to 10 years after planting (Aubert et al., 1984; Aubert, 1990; Gottwald et al., 1991; Roistacher, 1996).

It is estimated that close to 100 million trees are affected by HLB worldwide. In the northern and eastern regions of Thailand, 95 percent of trees were affected as of 1981. In the Philippines, HLB reduced the citrus acreage by 60 percent between 1961 and 1970. In Java and Sumatra, 3 million trees were destroyed from 1960 to 1970, and Bali lost 3.6 million trees from 1984 to 1987. In the southwestern oases of Saudi Arabia, HLB had killed most sweet orange and mandarin trees by 1983. Reunion Island lost its entire citrus industry in the 1960s due to HLB. In São Paul State, Brazil, where HLB was recognized in March 2004, close to 3 million HLB-affected sweet orange trees have been removed since mid-2004 in the HLB-control program. In Florida, where HLB was reported in 2005, intensive control measures were advocated but not implemented, except for some notable private efforts. By 2008, the situation had become so serious that the very existence of citrus is endangered. South Africa suffered devastating losses due to HLB at the time when the insect vector of this disease was not yet seriously controlled with systemic insecticides. Citrus was decimated in the northern part of the country (i.e. the White River region) while in the Tzaneen region, citrus has been replaced by avocado; the Hazy View area has lost 90 percent of its citrus; and many other regions are riddled with HLB (H. Le Roux, Citrus Research International, Nelspruit, South Africa, personal communication). However, since the late 1980s, when efficient control of the African psyllid was achieved with systemic insecticides, HLB in South Africa has become much less aggressive than the HLB in Brazil, Florida or Cuba. Because the African form of HLB is heat sensitive, the disease is severe in cool, elevated areas, whereas in some dry, hot areas, the disease is mild or absent.

General reviews on HLB and its vectors have been prepared by Bové, (2006, 2009), da Graça, (1991), da Graça and Korsten, (2004), Garnier and Bové, (1993), Gottwald et al., (2007), and Halbert and Manjunath, (2004).

History of HLB

In South China, in 1919, Reinking mentioned "yellow shoot" as a citrus disease of little importance (Reinking, 1919). By 1936, the "yellow shoot disease" had become a serious problem to which Kung Hsiang Lin, a phytopathologist at the South China Agricultural University, devoted most of his activities from 1941 to the late 1950s. From his discussions with the farmers in the Chaozhou district, Guangdong province, he learned that the disease had been in the Chaozhou district since the late 19th century and that the disease in South China probably originated from that district (Lin, 1956b). Subsequently, similar diseases were reported in citrus elsewhere in the world, e.g., in the Philippines in 1921, and in South Africa in the late 1920s. However, observed symptoms were attributed to mineral deficiencies (Van der Merwe and Andersen, 1937).

K. H. Lin was the first to report in 1956 that HLB is transmissible by graft-inoculation (Lin, 1956b). Professor A. Ciccarone, an Italian phytopathologist, published a report of Lin's work in 1957 in an Italian journal of citriculture (Ciccarone, 1957). In spite of this report, Lin's achievements remained largely unknown in the western world. Transmission of South African greening by graft-inoculation was reported nine years later, in 1965 (McClean and Oberholzer, 1965b). Transmission of the greening agent by the African citrus psyllid vector, *Trioza erytreae*, was also published in 1965 (McClean and Oberholzer, 1965a). Transmission of the HLB agent by the Asian citrus psyllid vector, *Diaphorina citri*, was reported simultaneously in 1967 in India (Capoor et al., 1967) and in the Philippines (Martinez and Wallace, 1967).

Citrus in India has been known to suffer seriously from certain disorders resulting in low production, twig dieback, slow death, and even sudden wilting. These symptoms were attributed to "dieback", and might have been observed by Roghoji Bhonsale (Capoor, 1963) in the 18th century, as well as by Bonavia (1888) in Assam. However, "dieback in citrus is not a specific disease" (Asana, 1958) and, over the years, many factors, including soil disorders, nutritional deficiencies, twig fungi, and viruses such as CTV, were evoked to account for it (Capoor, 1963).

Support for a connection between HLB and dieback came in 1966 from a survey on dieback in all major citrus areas of India. Researchers concluded that dieback was caused by the "virus" responsible for greening in South Africa (Fraser and Singh, 1968). Unquestionable proof for the presence of HLB in India came in 1967 when successful transmission of the disease agent with the Asian citrus psyllid was obtained (Capoor et al., 1967).

When first observed in South Africa in the late 1920s, HLB was referred to as "yellow branch" in the western Transvaal and as "greening" in the eastern Transvaal. The term "greening" prevailed and became the most common name of the disease worldwide. It refers to fruits with abnormal coloration (inverse coloration) in that they are still greenish at the stylar end, when the peduncular end has already turned orange/yellow; in normal fruits, coloration starts at the stylar end (Figure 2-9). In the Chaozhou district of southern China, where a local Chinese dialect was spoken, the disease was called huang long bing ("bing" meaning disease, "long" meaning shoot [and not "dragon"], and "huang" meaning yellow). Yellow shoot is an early, characteristic symptom of the disease and refers to the yellow color of the new flush of growth on infected trees. K. H. Lin referred to this disease as "huanglongbing" (Lin, 1956a). For these reasons, the International Organization of Citrus Virologists (IOCV) proposed in 1995 at the 13th conference of the IOCV in Fuzhou (Fujian province, China) that the official name of the disease be huanglongbing, and this proposal was accepted. Today, HLB is widely used for the African, American, and Asian forms of the disease.

Other names of the disease exist: "likubin" in Taiwan; "blotchy mottle disease" in South Africa, a very good name referring to the most characteristic leaf symptom (McClean and Schwarz, 1970); in the Philippines, the names "leaf mottling" (Salibe and Cortez, 1968) and "leaf-mottle-yellows" (Martinez and Wallace, 1968) also refer to leaf mottle, but lack the important term "blotchy"; in India, "citrus decline" or "citrus dieback" are unspecific names, as disorders other than HLB are often involved in citrus trees affected by decline or dieback; "vein-phloem degeneration" in Indonesia, draws attention on an important histological symptom.

Bacterial Agents Associated with HLB

Initially, it was thought that the HLB agent was a virus because of its graft transmissibility (Lin, 1956b). In 1967, Doi et al. in Japan, showed that "mycoplasmas" (today referred to as "phytoplasmas," plant-infecting bacteria that lack cell walls) were associated with certain plant diseases and that those diseases could be graft transmitted. Previous to this development, mycoplasmas were known mostly as human and animal pathogens. Because of the discovery of phytoplasmas, the presumed viral etiology of many plants diseases was re-examined, including HLB.

In 1970, through the use of electron microscopy (EM), Laflèche and Bové reported the presence of bacteria-like structures in the sieve tubes of HLB affected sweet orange seedlings from Africa and Asia (Laflèche and Bové, 1970a, b). First described as mycoplasma-like or having no cell wall, the bacteria-like structures were later on shown by Saglio et al. (1971) to clearly possess a cell wall. Moll et al. (1974) in South Africa also drew attention to the similarity between the cell-wall of the HLB-associated agent and Gram-negative bacteria (i.e having a cell wall composed of an outer membrane and an inner peptidoglycan layer). The Gram-negative nature HLB-associated organism was finally demonstrated in 1984 (Garnier et al., 1984a, b). . Most HLB-affected citrus trees worldwide carry one of three recognized liberibacter species designated as *Candidatus* Liberibacter africanus (*C*Laf), *Ca.* Liberibacter asiaticus (*C*Las), or

Ca. Liberibacter americanus (*C*Lam). However, in early 2007, a genuine plant mycoplasma, i.e., a phytoplasma, belonging to 16Sr group IX was detected in sweet orange trees in northern São Paulo State (Teixeira et al., 2008a). Another HLB-associated phytoplasma, belonging to a different group (16Sr group I) than the one detected in São Paulo State, has been identified in the Guangdong province of southern China (Chen et al., 2009).

Phytoplasmas can be easily distinguished from the walled liberibacters by EM. The symptomatic leaves from these trees in Saõ Paulo tested negative for all three liberibacter species, but were eventually found to be infected with a sieve-tube restricted phytoplasma which has 99 percent 16SrDNA sequence identity with the pigeon pea witches' broom phytoplasma (group 16Sr IX) (Teixeira et al., 2008a). The insect vector of the HLB phytoplasma in São Paulo State has not yet been identified. *Crotalaria junceae* plants, grown in between citrus rows for soil improvement and showing characteristic witches' brooms, have been found to be infected with the HLB phytoplasma (Wulff et al., unpublished). Thus, a non-citrus host is probably the source of phytoplasma inoculum on which potential insect vectors, such as leafhoppers, planthoppers or psyllids, may become infected and transmit the phytoplasma to citrus. In summary, symptoms indistinguishable from those induced by *C*Las infection have also been shown to be induced by specific phytoplasmas in the absence of *C*Las or other liberibacters.

Liberibacters and the phytoplasmas have not been available as pure (axenic) cultures. Thus, the criteria for establishing causality (Koch's postulates) could not be fulfilled, and it could not be demonstrated that bacteria of either of these groups were indeed causal agents of HLB. In general, the three liberibacters and the two phytoplasmas should not be called 'agents", "causal agents", or "pathogens" of HLB. Rigorously, they should be designated as "agents" or "bacterial agents" associated with HLB. However, there is strong circumstantial evidence that these associated agents, and in particular the liberibacters, are in fact etiological agents of HLB. The term "*Candidatus*" preceding a Latin binomial name indicates that the corresponding bacterium is not available in axenic culture and has only been associated with HLB by DNA sequence-based and other techniques (Murray and Schleifer, 1994).

Because axenic cultures were unavailable in the 1990s, comparisons with known bacterial 16S rDNA sequences from data bases were employed to show that the Asian and African HLB-associated organisms are indeed Gram-negative bacteria, and, more precisely, are members of a new subgroup in the alpha subdivision of the *Proteobacteria*. Organisms in this subdivision live in intimate association with eukaryotic cells and, in many cases, have the ability to survive and grow within an arthropod vector. The HLB-associated bacterium fits this description remarkably well because it grows in a specialized niche in its eukaryotic plant host, the phloem sieve tubes, and is transmitted by two arthropod vectors, the citrus psyllids, *T. erytreae* (the vector in Africa) and *D. citri* (the vector in Asia), in which it circulates and replicates.

Because the HLB-associated bacterium was the first representative of a new subgroup in the alpha-*Proteobacteria*, a new name was coined: "Liberobacter" from the Latin liber = bark and bacter = bacterium (Jagoueix et al., 1994). Liber<u>o</u>bacter was later replaced by Liber<u>i</u>bacter (Garnier et al., 2000a). The HLB-associated agent from Africa, *C*Laf, can be distinguished from the agent in Asia, *C*Las, on the basis of temperature sensitivity (Bové et al., 1974), as well as nucleotide sequence (Villechanoux et al., 1993), and serology (Garnier et al., 1991; Gao et al., 1993; Jagoueix et al., 1994; Garnier et al., 2000b). Sequence identification of the region between the 16S rRNA gene and the 23S rRNA gene (16S/23S intergenic region) has confirmed the notion that the African liberibacter and the Asian liberibacter represent two different liberibacter species (Jagoueix et al., 1997; Subandiyah et al., 2000).

A third liberibacter species (American HLB-associated bacterium) has been identified in São Paulo State, Brazil, shortly after HLB was detected there in 2004. Comparison of the sequences of the 16S rDNA and the 16S/23S intergenic regions of *C*Laf, *C*Las, and the American HLB-associated bacterium indicated that the latter is a new species: *Ca.* Liberibacter americanus (*C*Lam) (Teixeira et al., 2005a; Teixeira et al., 2005e; Teixeira et al., 2005d; Teixeira et al., 2005b). Transmission by graft-inoculation to healthy sweet orange seedlings has been demonstrated and EM observations showed *C*Lam to be restricted to the sieve tubes. The host(s), characteristics, distribution, and natural vectors of all three Liberibacters are shown in Table 2-2.

Liberibacter associated with non-citrus hosts**.** The potato zebra chip (ZC) disease, so-called because chips from afflicted tubers exhibit stripes when they are fried, was first reported in Mexico in 1994. Not long after that, in 2004, the disease was reported in Texas, near Pearsall (Secor and Rivera-Varas, 2004). In recent years (2004–2006), ZC has become an economically important disease in the United States and in Mexico, with losses amounting to millions of dollars. Two reports released within months of each other, one from a group in California (Hansen et al., 2008) and the other from a group in New Zealand (Liefting et al., 2009), described the association of a liberibacter with ZC and another disease that occurs in tomato, called psyllid yellows. Two names have been proposed for this liberibacter, *Ca.* Liberibacter psyllaurous (Hansen et al., 2008) and *Ca.* Liberibacter solanacearum (Liefting et al., 2009); both are transmitted by the potato/tomato psyllid, *Bactericera cockerelli*. *B. cockerelli* is associated with diseases of solanaceous plants, tomato (*Solanum lycopersicum*), potato (*S. tuberosum*), tamarillo (*S. betaceum*), capsicum (*Capsicum annuum*), chilli (*Capsicum* sp.), and cape gooseberry (*Physalis peruviana*) in New Zealand (Liefting et al., 2009) and with the potato ZC in the United States (Lin et al., 2009)**.** The liberibacter associated with ZC is the first liberibacter to have been described from non-citrus hosts and was found to have 95–97 percent 16S rDNA sequence identity with the HLB-associated liberibacters. The names "*Ca.* Liberibacter psyllaurous" and "*Ca.* Liberibacter solanacearum" are probably synonymous based on the currently available 16S rDNA sequences from the National Center for Biotechnology Information (NCBI), and are both included in the List of Prokaryotic names with Standing in Nomenclature (last updated on March 6, 2010). However, names included in the category *Candidatus* are not covered by the Rules of the Bacteriological Code (1990) (for naming new bacterial taxa) since these are only candidate species not actual species. Accordingly, a name included in the category *Candidatus* cannot be validly published. Therefore, the bacterium is best referred to, at present, as "*Ca.* Liberibacter psyllaurus/solanacearum" (*C*Lp/s). Citrus is not a host of the potato/tomato psyllid. However, work by Garnier and Bové (1983) and Duan et al. (2008), which demonstrated *C*Las transmission to, and symptom production in tobacco (*Nicotiana tabacum*) and tomato using dodder (*Cuscuta campestris*, a parasitic plant), suggests that there is the possibility that the liberibacter found in potato can also be dodder-transmitted to, and can produce symptoms in, citrus (sweet orange). Once in citrus, *C*Lp/s could be acquired and inoculated by the ACP. In other words, it is not excluded that *C*Lp/s might behave, once it gets into citrus, as another HLB liberibacter.

TABLE 2-2 Bacterial Agents Associated with Huanglongbing

Agent	Host(s)	Characteristics	Distribution	Natural Vector	References
HLB Associated Liberibacters					
Candidatus Liberibacter africanus	*Citrus* spp.; transmissible to periwinkle (*Catharanthus roseus*) by dodder (*Cuscuta campestris*)	Sieve-tube restricted; gram-negative; heat sensitive	Africa; Arabian Peninsula; Mauritius; Reunion Islands	*Trioza erytreae* (African citrus psyllid)	Garnier and Bové, 1983; Jagoueix et al., 1994; Garnier et al., 2000a
Candidatus Liberibacter americanus	*Citrus* spp.; transmissible to periwinkle by dodder	Sieve-tube restricted; gram-negative; heat sensitive	Brazil (São Paulo and Minas Gerais); Hunan, China (unconfirmed)	*Diaphorina citri* (Asian citrus psyllid)	Teixeira et al., 2005a, b,d,e; Lopes et al. 2009
Candidatus Liberibacter asiaticus	*Citrus* spp.; transmissible to periwinkle, tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*) by dodder	Sieve-tube restricted; gram-negative; heat tolerant; causes disease at 32–35C	Asia; Saudi Arabia; Florida; Brazil	*Diaphorina citri* (Asian citrus psyllid)	Bové et al., 1974; Garnier and Bové, 1983, 1993; Jagoueix et al., 1994, 1997; Garnier et al., 2000a; Duan et al., 2008
Liberibacters from Non-rutaceous Hosts					
Candidatus Liberibacter psyllaurous	Solanaceous crops	97% sequence similarity with *Candidatus* Liberibacter asiaticus; causes zebra chip disease in potato	Mexico; Texas; Guatemala	*Bactericera cockerelli* (potato/tomato psyllid)	Gudmestad and Secor, 2007; Hansen et al., 2008; Lin et al., 2009
Candidatus Liberibacter solanacearum	Tomato; Chili; Pepper; Potato; Tamarillo; Cape Gooseberry	Likely the same as *Candidatus* Liberibacter psyllaurous	New Zealand	*Bactericera cockerelli* (potato/tomato psyllid)-possibly	Liefting et al., 2009a, b

Agent	Host(s)	Characteristics	Distribution	Natural Vector	References
Phytoplasma Associated with HLB					
Phytoplasma	Citrus; *Crotolaria juncea*	Sieve-tube restricted; wall-less; has 99% sequence identity with pigeon pea witches' broom phytoplasma (group 16Sr IX)	Brazil (San Paulo)	Not yet known	Teixeira et al.., 2008c; Wulff et al.., 2009
Phytoplasma	Citrus	Sieve-tube restricted; wall-less; related to *Candidatus* Phytoplasma asteri (group 16Sr I)	China (Guangdong)		Chen et al.., 2009

Transmission

HLB is readily transmitted (acquired and inoculated) by budwood or by insect vectors, i.e., the African psyllid, *T. erytreae* (McClean and Oberholzer, 1965a) or ACP (Capoor et al., 1967; Martinez and Wallace, 1967). Low efficiency seed transmission of *C*Las has been reported (Zhou et al., 2008) but needs to be quantitated by further studies. Attempts at mechanical inoculation by introducing sap from infected trees into test plants proved to be unsuccessful. Graft inoculations of *C*Lam with shoots, buds, bark from shoots or roots, and leaf patches has been successful with varying efficiency, depending on the species and the size of the tissue used (Lopes and Frare, 2008). Transmission by dodder from citrus to periwinkle, tobacco, and tomato (*Lycopersicon esculentum*) has been demonstrated by Garnier and Bové (1983), Garnier and Bové (1993), and Duan et al. (Duan et al., 2008), respectively.

Transmission by psyllid is considered as the primary mode of HLB spread in the field. The following section provides a brief summary of what is currently known about ACP life history and biology. More information can be found in a paper presented by Hall (2008) at the 2008 North American Plant Protection Organization (NAPPO) Workshop on Citrus Huanglongbing and the Asian Citrus Psyllid (held in Hermosillo, Sonora, Mexico) and in a review by Halbert and Manjunath (Halbert and Manjunath, 2004).

ACP description, life history, and biology

The ACP is 2.7 to 3.3 mm in length and has mottled brown wings and piercing mouthparts (stylets) which are used to ingest phloem sap from young citrus stems and leaves. Adults resting or feeding on leaves are found with their heads low and their bodies at a 45° angle with the plant surface (Figure 2-7, A and D). They are active and are capable of jumping or flying short distances when disturbed (Hall, 2008). The ACP life cycle includes an egg stage (Figure 2-7, B) and five nymphal instars (Figure 2-7, C) which take 11–15 days to complete (Chavan and Summanwar, 1993).

Oviposition and development of nymphs occur on young, tender flush leaves or shoots (Hall and Albrigo, 2007). ACP developmental times vary with temperature, with 25–28°C as the optimum temperature range for development (Liu and Tsai, 2000). Adults reach reproductive maturity 2 or 3 days after emergence, and oviposition (egg laying) begins 1 or 2 days after mating (Wenninger and Hall, 2007). Females were observed to oviposit throughout their lifetime if young leaves were present. According to reports from Husain and Nath (1927), Pruthi and Batra (1938), Tsai and Liu (2000) and Nava et al. (2007), the adult females are capable of laying 500 to 800 eggs or more over a period of two months. Studies by Skelly and Hoy (2004) indicated that oviposition by adult ACP is influenced by temperature and by relative humidity; exposure to 34° C for 5 days caused adult ACP to stop laying eggs and fewer eggs were produced when relative humidity dropped below 40 percent (Skelley and Hoy, 2004).

Maximum adult longevity ranged from 117 days at 15°C to 51 days at 30°C (Liu and Tsai, 2000); survival was observed to increase with increasing humidity (McFarland and Hoy, 2001). Very low temperatures have an adverse effect on ACP; 94 to 98 percent mortality was observed in adults that were kept at -3.3°C (Ashihara, 2004). ACP development, longevity, and reproduction also vary depending on the host species (Tsai and Liu, 2000; Fung and Chen, 2006;

FIGURE 2-7 Asian citrus psyllid (ACP) life cycle. Clockwise from top left: A) ACP adult and nymphs; B) eggs of the ACP; C) five nymphal stages of the ACP; and D) closer view of ACP mottled brown wings and 45° angle with plant surface.
Source: (A) Courtesy of M. E. Rogers, Citrus Research and Education Center, Lake Alfred, FL; (B, C, and D) Courtesy of David Hall, USDA-ARS-USHRL, Fort Pierce, FL.

Nava et al., 2007). Studies by Tsai and Liu (2000) showed that the psyllid developed faster on grapefruit than on rough lemon, sour orange, or orange jasmine (Tsai and Liu, 2000).

Wenninger et al. (2008; 2009) observed that male and female courtship behavior includes substrate-borne vibrational signals; they also found behavioral evidence that the female ACP emits a sex pheromone (Wenninger et al., 2008). Mating, which is restricted to daylight hours (Wenninger and Hall, 2007), has been found to occur multiple times with different partners (Wenninger and Hall, 2008). Oviposition and other mobile activities of the psyllid were also observed to occur only during daylight hours (Wenninger and Hall, 2007). Aubert and Hua (1990) noted that flight activity is more pronounced during warm, windless, and sunny afternoons between the hours of 4 and 6 pm. Research in Florida indicate that low numbers of adults routinely disperse from citrus within distances of 8 to 60 m (Hall, 2008) with some evidence for occasional mass migrations (Hall et al., 2008).

Population fluctuations of ACP are strongly influenced by the availability of young citrus flush, since eggs are laid only on young flush where nymphs hatch and develop. Large infestations of ACP occur during late spring through midsummer, but outbreaks can occur at any time of the year depending on warm temperatures and availability of young flush leaves (Hall, 2008). In India, Husain and Nath (1927) recorded nine ACP generations on citrus over a one year period and they speculated that two or more additional generations can occur in warmer weather with ample flush available.

ACP host plants

Halbert and Manjunath (2004) provide a list of plant species that are hosts of the ACP. The psyllids can feed on many citrus species and citrus close relatives, but the preferred hosts are *Murraya paniculata* (Orange jasmine, mock orange) (Aubert and Quilici, 1988) and *Citrus aurantifolia* (Halbert and Manjunath, 2004). However, studies done by Tsai and Liu (2000) and Tsai et al. (2002) did not show the psyllid's preference between grapefruit and orange jasmine. Continuous shoot growth by *M. paniculata* plays an important role in maintaining ACP populations when citrus flush is not available (Tsai et al., 2002).

Vector-pathogen interactions

There are a number of reports on the transmission of *Ca.* Liberibacter spp. by ACP (Capoor et al., 1974; Huang et al., 1984; Xu et al., 1988; Hung et al., 2004; Brlansky and Rogers, 2007) that together support a horizontal (plant-to-plant), circulative-propagative transmission mechanism. Nymphs appear to acquire the pathogen during later instar stages; adults emerging from these nymphs can immediately inoculate trees with the pathogen. Studies by Xu et al. (1988) indicated that the first, second, and third instars cannot inoculate the pathogen but the fourth and the fifth instars are able to do so. Adults can acquire the pathogen with feeding times ranging from 30 minutes to 5 hours (Capoor et al., 1974; Xu et al., 1988). Since nymphs have limited mobility and spend their entire time feeding in one small area of a tree, they may be the most important stage for efficient acquisition. The adult stage with its mobility, however, is the stage which spreads the pathogen from tree to tree.

The transmission cycle of HLB includes the acquisition, retention (after a latent period), and inoculation of the pathogen by the vector. In general, pathogen transmission by the vector occurs during feeding and involves the bacteria traversing a complicated path through the vector. However, the mechanism of pathogen transmission is poorly understood for psyllid-Liberibacter interactions, and research is underway to decipher critical processes involved in this pathway. After the pathogen is acquired, it multiples in the insect, and the vector remains infected (inoculative) for life. It is reported that a latent period of up to 25 days occurs after acquisition but before the adult can inoculate the pathogen (Xu et al., 1988; Hung et al., 2004). Capoor et al. (1974), however, reported a latent period of only 8–12 days. ACP was shown to inoculate the plant during a feeding period of 5 hr (Xu et al., 1988), but results derived from modern techniques could alter this timeframe. Transmission of *C*Las is being investigated by electrical penetration graphs to relate psyllid feeding behavior to both acquisition and inoculation (Bonani et al., 2010). Investigations of transovarial transmission have so far given only negative results (Capoor et al., 1974; Xu et al., 1988; Hung et al., 2004), and effects of *C*Las on the life-span or fecundity of the psyllid have not been reported. Many early reports from Asia indicate low transmission efficiency (ranging from 1.3 to 12 percent) by ACP (Huang et al., 1984; Xu et al., 1985; Xu et al., 1988). However, field infection rates of 50 to 70 percent have been observed in young citrus groves in China (Xu et al., 1988).

ACP distribution

The ACP distribution is discussed in another section of this chapter (see *Worldwide Distribution*).

Epidemiology

The ACP was first detected on *Murraya* in Palm Beach County, Florida in 1998 and became widespread in Florida as a result of shipment of infested orange jasmine (Halbert and Manjunath, 2004). *Murraya* spp. has been shown to be a host of the HLB pathogen but its role or importance in HLB epidemics is unknown. Based on observations of localized HLB secondary spread, much movement is presumed to be of short distance. However, when HLB was first diagnosed in 2005 in coastal communities in Miami-Dade County, the disease was soon found in a commercial grove some 90 to 150 km away. Gottwald et al. (2007) speculated that this could have resulted from long range dispersal of infected psyllids. In any event, the pathway of long-distance pathogen spread likely involves infected psyllids migrating from citrus to citrus as well as hitchhiking on infested ornamental plants such as *Murraya* , its preferred host.

The epidemiology of HLB is difficult to assess due to the fastidious nature of the presumed causal agent, its persistent/propagative mode of transmission by psyllid vectors, the long latent period needed for symptom development, as well as variability of HLB symptoms due to climate, horticultural practices and different citrus varieties. This often leads to disparate observations in estimates of disease incidence. The recent use of polymerae chain reaction (PCR) to accurately diagnose HLB infection in trees and psyllids has vastly improved this problem (Gottwald et al., 2007) by its ability to confirm infection of asymptomatic or questionable trees.

Symptomatology

Symptoms of infected field tree

In sweet orange trees, the early stages of infection can be identified by the presence of one or several characteristic “yellow shoots”. With time, the yellow shoots grow into larger yellow/pale green branches. In South Africa, trees with large yellow branches are characteristic of HLB. In later stages of the disease, the yellow branches take over the whole canopy, indicating that the tree is fully (systemically) infected. In São Paulo State, the “yellow branch” stage is less characteristic of HLB (Bové, 2009). Affected branches may show one or several of the following features: defoliation, twig dieback, presence of leaves with blotchy mottle (see below), mineral deficiency patterns (zinc in particular), or uniformly yellow, presence of affected fruits, which have a tendency to drop. Defoliation results in sparse foliage and an “open” type of growth. In Brazil, Florida, and Cuba, many young orchards, because of abundant growth flushes, became affected more severely than older orchards with fewer flushes. While many trees in these young orchards were fully affected, other trees still had some symptomless sectors with well-developed green leaves, while the symptomatic sectors had yellowish leaves with severe zinc deficiency patterns. Such trees, with symptomless sectors and symptomatic sectors, are most characteristic of orchards being invaded by HLB. In fall and early winter, these trees have blotchy mottle leaves, which may drop in late winter and make HLB diagnosis more difficult.

In mandarin trees, HLB is characterized by yellowing of all sectors of the canopy and severe defoliation. HLB-affected Murcott tangor trees in São Paulo State have pale green/yellowish branches, undergo defoliation and show severe fruit symptoms.

Symptoms on leaves

One of the most characteristic symptoms of HLB, worldwide, is leaf “blotchy” mottle. Leaves with blotchy mottle have several shades of yellow, pale green and dark green. These shades blend into each other, and there are no sharp boundaries between the various shades of color, hence the term "blotchy" mottle (Figure 2-8). Blotchy mottle is synonymous with HLB and accompanies HLB wherever it occurs in the world. Zinc, manganese or magnesium deficiencies also produce a blotchy mottle but with a symmetrical pattern on the two sides of the leaf unlike the asymmetric pattern characteristic of HLB. The only other disease that produces similar symptoms is lime blotch/wood pocket, a genetic disorder of Tahiti or Persian limes (Timmer et al., 2000), and those symptoms occur exclusively on limes. With trees in early stages of HLB, blotchy mottle may affect large, well-developed leaves, and may be the only leaf symptom to be seen. In later stages, foliar symptoms of zinc deficiency will eventually develop, and such leaves will remain small, and with time, the whole leaf blade may ultimately turn uniformly yellow. Leaves may become thicker and leathery. Midribs and lateral veins are sometimes enlarged, swollen, and corky. In Florida, yellow midribs are prevalent on HLB-affected leaves.

FIGURE 2-8 Huanglongbing symptoms on citrus trees (left) and leaves on a branch (right). Source: Photos courtesy of M. Irey, US Sugar Corporation, Clewiston, FL.

Blotchy mottle is most apparent on sweet orange leaves, but most other citrus species and varieties also show it, including grapefruit, pummelo, citron, rough lemon, *Citrus macrophylla,* Volkamer lemon, and sour orange. Leaves of lemon, Mexican lime, and Tahiti lime also show blotchy mottle, but of a type slightly different from that of sweet orange leaves, as the patches of dark green tend to be much larger than in the case of sweet orange leaves. Blotchy mottle symptoms also vary among the mandarin varieties that are grown in different regions of the world.

Symptoms on fruit

HLB also produces characteristic symptoms on fruit, which are easily seen on sweet oranges but are also observed on mandarins, tangors, pummelos, and many other species, hybrids or varieties. In normal fruits, the orange color develops first at the stylar end, at a time when the peduncular end is still green. On HLB-affected fruit, the orange color starts first at the peduncular end, at a time when the stylar end is still green. Fruits affected by HLB also exhibit orange-stained vascular bundles, are lopsided and are smaller than normal fruit, and have aborted seeds (Figure 2-9). Juice from fruit displaying these symptoms is similar in quality to juice from immature fruit. However, trees affected with HLB are also characterized by severe drop of symptomatic fruits. Most symptomatic fruit either drops before harvest, is not picked, or is eliminated by the sizing equipment. Therefore, under current conditions, HLB severely affects yields of juice oranges but has not presented a significant quality problem for the juice.

Host Range

Non-rutaceous hosts

It has been demonstrated that the three citrus liberibacters can be transmitted to periwinkle plants by dodder (Garnier and Bové, 1983; Bonnet, unpublished). Dodder-transmission of *C*Las to tobacco (Garnier and Bové, 1983) and to tomato (Duan et al., 2008) has also been demonstrated, indicating that *C*Las can infect solanaceous plants.

Sensitive species, varieties or scion-rootstock combinations (Citrus and Citrus relatives)

The most severe symptoms are found on sweet orange, mandarin, tangelo, and grapefruit, followed by lemon, rough lemon, and sour orange. Severe fruit and leaf symptoms can be seen on pummelo (*Citrus grandis*), even though this species is sometimes erroneously considered more or less tolerant. Trees of small-fruited, acid lime (*C. aurantifolia*) are only slightly affected, but clear-cut blotchy mottle symptoms can be seen on leaves. In many countries where HLB occurs, CTV is also present and affects acid lime much more severely than does HLB. However, the lime trees are severely affected by the ACP, which is a significant pest for lime trees.

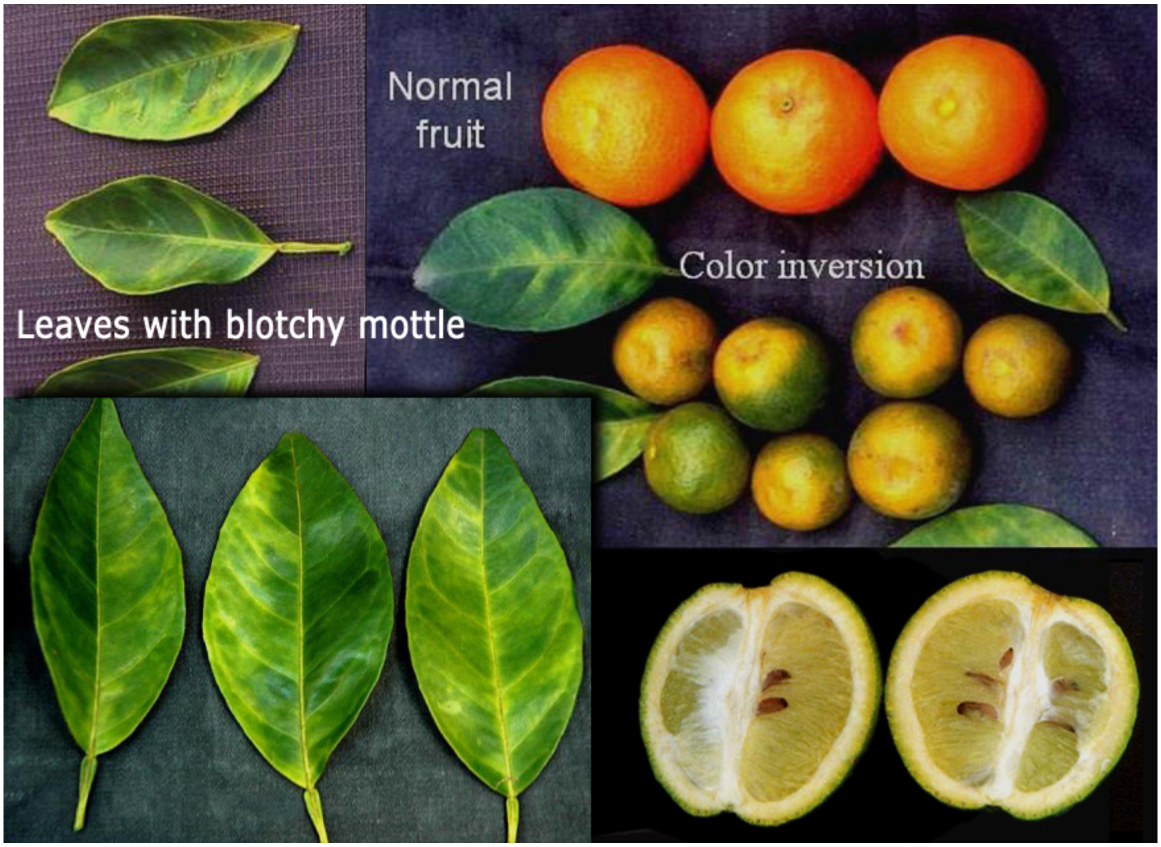

FIGURE 2-9 HLB symptoms on citrus leaves and fruit. Lopsided fruit with aborted seeds and brownish vascular bundles (lower right).
Source: Courtesy of J. M. Bové, La Brède, France.

The ornamental citrus relative, *M. paniculata* , shows leaf yellowing, defoliation and dieback on branches when infected with *C*Las or *C*Lam in São Paulo state (Lopes et al., 2005; Lopes et al., 2006). *M. paniculata* was also found in Florida to be naturally infected with the *C*Las (Zhou et al., 2007). Leaves of *Clausena lansium* (Chinese wampee) infected with *C*Las show leaf mottle (Ding et al., 2005). *Severinia buxifolia* (Chinese box orange) is also a host of *C*Las (Hung et al., 2000).

In the Western Cape Province of South Africa, *Calodendron capense*, an ornamental rutaceous tree (Cape chestnut tree), showed blotchy mottle leaves, and was found to be infected with a liberibacter. The new liberibacter was characterized as subspecies "capensis" of *Ca.* L. africanus (Garnier et al., 2000a).

Symptomless species, varieties or scion-stock combinations

There are no citrus species, varieties or combinations that are immune to HLB; trifoliate orange (*Poncirus trifoliata*) is said to be tolerant, but citranges may show leaf symptoms.

Worldwide Distribution

For many years, HLB was restricted to two large geographical regions: Africa and Asia, with the Arabian Peninsula between. At present, HLB occurs in the following regions of the world: Asia, Southeast Asia, Oceania, Africa, Madagascar, Arabian Peninsula, Reunion and Mauritius Islands, and America. The distribution of the HLB-associated liberibacters and the HLB insect vectors are given in Table 2-3 and Figure 2-10.

In all countries in Asia, Southeast Asia, and Oceania, the HLB-associated agent and the psyllid vector are temperature tolerant. Symptoms of Asian HLB occur at temperatures up to 32–35°C, if not higher. In the African countries where HLB is found, as well as in Madagascar, the disease and the African psyllid vector are only present in cool, elevated areas with temperatures below ~30°C, as the African HLB-associated liberibacter (*C*Laf) and the African psyllid are both temperature sensitive (Catling, 1969; Bové et al., 1974). The African psyllid is also severely affected by relative humidities below 25 percent.

In Saudi Arabia, HLB and the ACP were present in the early 1980s in the western oases along the Red Sea from Mecca down to the border with Yemen. In Yemen, HLB and the African vector occurred only on cool uplands, the HLB-associated liberibacter and its vector being both of the temperature-sensitive type. At the Saudi/Yemeni border, the two psyllid vectors were found in the same orchards, and the two HLB-associated liberibacters, *C*Laf and *C*Las, were probably present too (Bové and Garnier, 1984).

T. erytreae and ACP, as well as *C*Laf and *C*Las, were present on the two Indian Ocean islands, Reunion and Mauritius (Garnier et al., 1996). On Reunion, *C*Las and ACP occur from sea level up to ~500m. Above 500m, the *C*Laf and *T. erytreae* predominate. On both islands, some sweet orange trees carry both *C*Laf and *C*Las.

HLB has been present in the Americas since 2004. The countries where the disease occurs are listed in Table 2-3. In some of these countries, the disease was presumably present for several years before being reported. *Ca.* Liberibacter asiaticus is present in North and Central America, and both *C*Lam and *C*Las occur in Brazil, with ACP as the vector.

The ACP has been present in the Rio Grande Valley of Texas (United States) since 2001 and in the Yucatan peninsula of Mexico since 2002. It quickly spread throughout Mexico, and, in June 2008, it was seen in Tijuana, at the border between Baja California, Mexico, and California, USA. By October 2008, it was detected in southern California along the United States/Mexico border. In October of 2009, ACP was detected in the Los Angeles basin and in Orange County (http://www.cdfa.ca.gov/phpps/acp/). It is present throughout the Southern United States, including Texas, Alabama, Georgia, Mississippi, and South Carolina and has been recently detected in Arizona. Table 2-3 lists regions that have the psyllid vectors but not HLB.

As of January 2009, HLB and two psyllid vectors have not been detected in the countries around the Mediterranean Basin, as well as Australia, New Zealand, and North- and South-Pacific islands, except that ACP is present on Hawaii and Maui.

Diagnosis

Diagnostic field symptoms. When HLB enters a region hitherto free of the disease, young trees show symptoms earlier, are more severely affected, and have a more rapid disease progression than adult trees. Young trees may be fully infected or still have some symptomless

Table 2-3 Geographical Distribution of Huanglongbing-associated Liberibacters and their Insect Vectors

Organism	Countries Present	References
	AFRICA	
Candidatus Liberibacter africanus and *Trioza erytreae*	Burundi	Garnier and Bové, 1996; Bové, 2006
	Cameroon	Garnier and Bové, 1996; Bové, 2006
	Central African Republic	Garnier and Bové, 1996; Bové, 2006
	Ethiopia	Garnier and Bové, 1996; Bové, 2006
	Kenya	Garnier and Bové, 1996; Bové, 2006
	Malawi	Garnier and Bové, 1996; Bové, 2006
	Nigeria	Garnier and Bové, 1996; Bové, 2006
	Somali	Garnier and Bové, 1996; Bové, 2006
	South Africa	McClean and Oberholzer, 1965a
	Swaziland	Garnier and Bové, 1996; Bové, 2006
	Tanzania	Garnier and Bové, 1996; Bové, 2006
	Zimbabwe	Garnier and Bové, 1996; Bové, 2006
	ARABIAN PENINSULA	
	Yemen	Bové and Garnier, 1984
	INDIAN OCEAN:	
	Madagascar Island	Bové, 2006
	ASIA	
Candidatus Liberibacter asiaticus and *Diaphorina citri*	Bangladesh	Bové, 2006
	Bhutan	Bové, 2006
	Cambodia	Bové, 2006
	China	Bové, 2006
	East Timor	Bové, 2006
	India	Capoor et al., 1967
	Indonesia	Bové et al., 2000a

Organism	Countries Present	References
	Iran	Faghihi et al., 2009
	Japan	Miyakawa and Tsuno, 1989
	Laos	Bové, 2006
	Malaysia	Miyakawa and Tsuno, 1989
	Myanmar	Bové, 2006
	Nepal	Bové, 2006
	Pakistan	Bové et al., 2000b
	Papua New Guinea	Bové, 2006
	Philippines	Martinez and Wallace, 1967
	Sri Lanka	Bové, 2006
	Taiwan	Bové, 2006
	Thailand	Schwarz et al., 1973
	Vietnam	Bové, 2006
	ARABIAN PENINSULA	
	Saudi Arabia	Bové and Garnier, 1984
	AMERICA	
	Belize	Citrus Growers Association pamphlet, 2009
	Cuba	Luis Pantoja et al., 2008
	Dominican Republic	Matos et al., 2009
	Mexico	NAPPO, 2009d
	Puerto Rico	NAPPO, 2009c
	Florida, United States	Halbert, 2005
	Georgia, United States	NAPPO, 2009a
	Louisiana, United States	Lemon and Harless, 2008
	South Carolina, United States	NAPPO, 2009

Organism	Countries Present	References
Candidatus Liberibacter asiaticus, *Candidatus* Liberibacter americanus, and *Diaphorina citri*	Brazil	Coletta-Filho et al., 2004; Teixeira et al., 2005a–e
	China (unconfirmed)	Lou et al., 2008
	INDIAN OCEAN	
Candidatus Liberibacter africanus, *Candidatus* Liberibacter asiaticus, *Diaphorina citri*, and *Trioza erytreae*	Reunion Island	Garnier et al., 1996
	Mauritius Island	Garnier et al., 1996
	ARABIAN PENINSULA	
	Saudi Arabia/Yemen border region	Bové and Garnier, 1984
Diaphorina citri	Argentina	Vaccaro, 1994; Augier et al., 2006
	Bahamas	Halbert and Núñez, 2004
	Bolivia	Bové, 2006
	Cayman Islands	Halbert and Núñez, 2004
	Costa Rica	Villalobos et al., 2005
	Guadeloupe	Étienne et al., 1998
	Honduras	Burckhardt and Martinez, 1989
	Oman	Al-Zadjali et al., 2008
	Puerto Rico	Halbert and Núñez, 2004
	Venezuela	Cermeli et al., 2000
	Virgin Islands (St. Thomas)	Halbert and Núñez, 2004
	Texas, United States	French et al., 2001

Organism	Countries Present	References
	Hawaii and Maui, United States	Conant et al., 2007
	Alabama, Georgia, Louisiana, Mississippi, South Carolina, United States	NAPPO, 2008
	California, United States	CDFA, 2009
	Arizona, United States	NAPPO, 2009b
Trioza erytreae	Portugal (Madeira Islands)	PLANT Protection Service of Portugal, 1994–12
	Spain (Canary Islands, Tenerife, La Gomera, La Palma, El Hierro)	González Hernández, 2003
	United Kingdom (Saint Helena)	EPPO-CABI, 1997

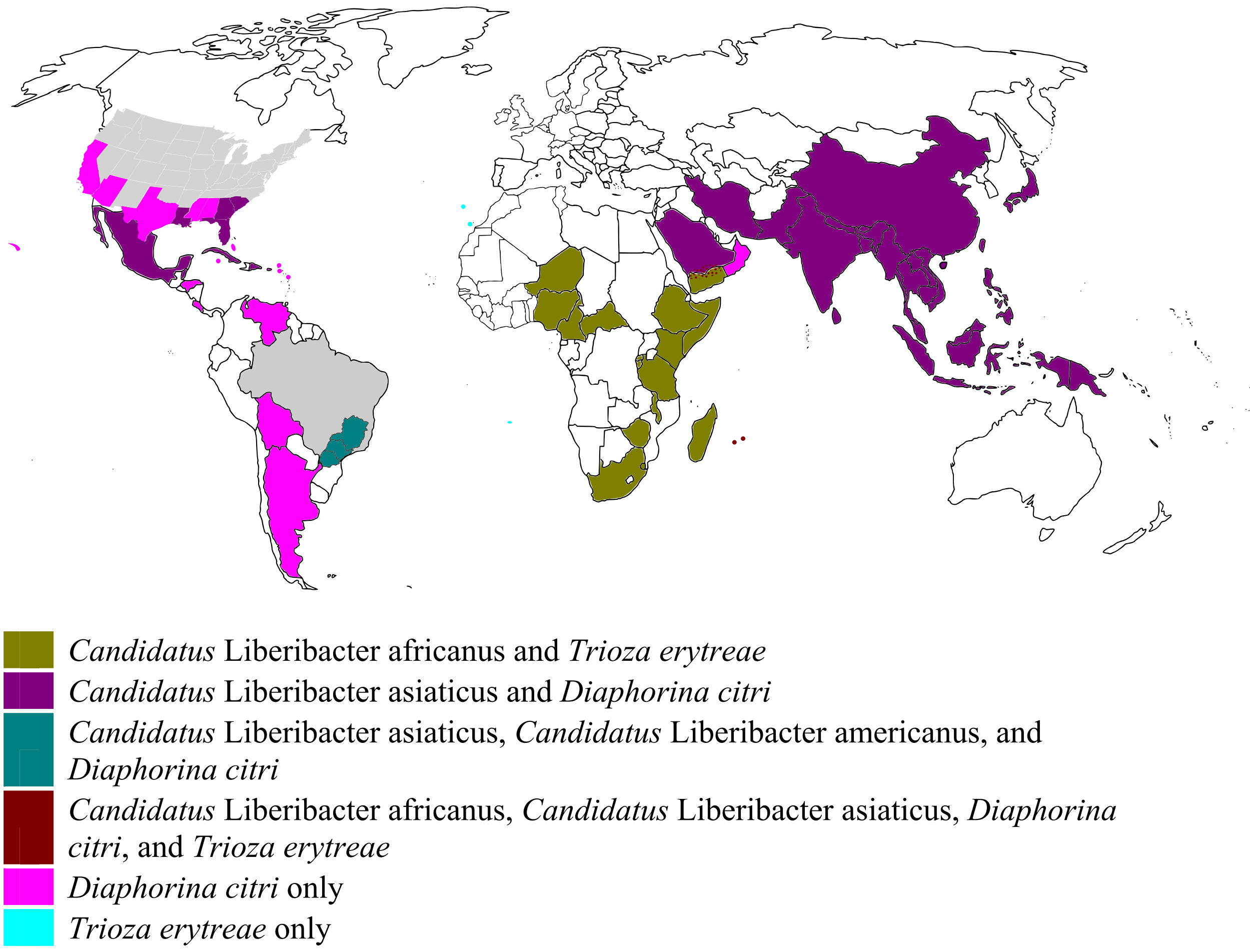

FIGURE 2-10 World distribution of *Candidatus* Liberibacter spp. and their insect vectors. Source: Based on Table 2-2.

sector(s). In general, trees are not uniformly affected. Diagnosis of HLB in the field is based on the characteristic symptoms that were discussed earlier in this section (see *Symptomatology*).

Biological indexing. Preferred indicator seedlings are sweet orange (such as Madam Vinous, Pineapple, Hamlin) and tangelo (Orlando, Seminole) cultivars. Inoculation can be done with budwood sticks, bark pieces, buds, and patches of blotchy mottle leaves from affected parts of the candidate tree. However, transmission is not 100 percent successful, probably because of low concentration and uneven distribution of the liberibacters, and 5 to 10 indicator seedlings should be used for each source to be indexed.

Electron microscopy. From 1970 to 1990, transmission electron microscopy (TEM) was the first and only laboratory technique for indisputable identification and confirmation of HLB and has been widely used (Garnier and Bové, 1996). The reliability and specificity of TEM is based on two properties of the HLB-associated liberibacter: (i) its exclusive location in the sieve tubes, and (ii) the presence of a cell wall (Figure 2-11). In citrus, no bacterium other than the HLB-associated liberibacters fit these criteria. However, TEM is a difficult and time-consuming technique, and it cannot distinguish between African, Asian or American liberibacters, but can distinguish between the walled liberibacters and the wall-less HLB-phytoplasmas.

FIGURE 2-11 Electron micrograph of *Candidatus* Liberibacter sp. in the phloem of infected citrus tree.
Source: J. M. Bové, La Brède, France.

Serological diagnostic methods. Thirteen monoclonal antibodies (MA), specific for the African or Asian liberibacters, have been produced (Garnier et al., 1991; Gao et al., 1993). The use of these MAs for the detection of the HLB liberibacters by immunofluorescence on thin sections has shown that each MA is very specific for the strain used for immunization and, therefore, it is not advisable to use the MAs, either singly or in cocktails, for generalized diagnosis of HLB (Garnier et al., 1991). They have, however, been used for purification of the liberibacter cells.

Molecular diagnostic methods. Today, molecular techniques, DNA hybridization and in particular PCR, are the techniques of choice for HLB diagnosis. The plant DNA required for these techniques is obtained from leaf midribs by such methods as the cetyltrimethylammonium bromide procedure of Murray and Thompson (1980). If possible, leaves with blotchy mottle symptoms should be used, as the liberibacter titer in these leaves is generally high (Teixeira et al., 2008b). The molecular diagnostic methods that have been used to detect the presence of HLB liberibacters include the following: DNA hybridization (see Villechanoux et al., 1992; Bové et al., 1993; Villechanoux et al., 1993; Planet et al., 1995; Teixeira et al., 2008b), conventional PCR (Jagoueix et al., 1996; Hocquellet et al., 1999; Teixeira et al., 2008b), nested PCR (Weisburg et al., 1991), and quantitative real-time PCR (Irey et al., 2006; Li et al., 2006; Wang et al., 2006; Li et al., 2007; Teixeira et al., 2008b).

Iodine-based starch test to assist in selecting leaves for HLB testing. Anatomical studies conducted in the 1960s found "massive accumulation" of starch in leaf samples collected from HLB-affected sweet orange trees. More recent studies have quantified starch accumulation in HLB-affected leaves at six times more than healthy leaves. Starch readily reacts with iodine, resulting in a very dark grey to black stain. Recently, a number of researchers from Vietnam and Japan have been working to adapt this starch/iodine reaction into a diagnostic tool for HLB, and they report up to 90 percent agreement between PCR analysis and starch tests with iodine. Researchers at the University of Florida Institute of Food and Agricultural Sciences (UF-IFAS) has not performed a similar correlation analysis, although studies are ongoing. An IFAS-developed version of this test, how to perform it, the required materials, its potential benefits, its limitations, and how to interpret the results has been reported (Etxeberria et al., 2007). The purpose of the test is to assist in determining which leaves, with difficult-to-interpret symptoms, should be submitted for PCR analysis. The test can be performed in the field but it does not replace PCR.

Management

Antibiotic treatments. Immediately after the discovery in 1970 that HLB is associated with a bacterium (and not a virus), tetracycline injections into the trunks of HLB-affected citrus trees were tried in South Africa, and found to significantly reduce the incidence of symptomatic fruit (Schwarz and von Vuuren, 1971; Schwarz et al., 1974; Moll and van Vuuren, 1977; Moll et al., 1980). The control procedure was, however, stopped after a few years because tetracycline is only bacteristatic and not bactericidal, requiring treatments to be repeated each year. After several trunk injections, the antibiotic induced phytotoxicity in the injected citrus trees. Tetracycline injections were used for some time in Taiwan (Su and Chang, 1974; Chiu et al., 1979) and Indonesia (Supriyanto and Whittle, 1991) without appreciable results. Wholesale use of an antibiotic in the orchard presents potential problems from development of antibiotic resistance. Experimentally, penicillin was shown to give remission of HLB symptoms, and this result supported the bacterial nature of the HLB agent (Aubert and Bové, 1980; Bové et al., 1980).

Approaches to biological control of psyllid vectors. The ACP in Florida is attacked by many generalist predators such as spiders, lacewings, syrphids, ladybugs, minute pirate bugs, along with a number of hymenopterous wasp parasitoids. However, the natural enemy reported most effective in reducing psyllid populations were coccinellid lady beetles (Michaud, 2004), including the larval and adult stage ACP predators *Olla v-nigram* (Mulsant) and *Harmonia axyridis*. Classical biological control, defined as the introduction of natural enemies from the pest's region of origin, has been implemented in Florida for ACP control. The tiny wasp parasitoids (so named because, unlike parasites, they always kill their hosts) *Tamarixia radiata* from Taiwan and Vietnam and *Diaphorencyrtus aligarhensis* (Shafee et al., 1975) from Taiwan were imported, reared and released against the ACP in Florida (McFarland and Hoy, 2001). Apparently, only *T. radiata*, a wasp that lays a single egg beneath the psyllid nympth and has shown some success against ACP in the islands of Guadaloupe (Étienne et al., 2001), has become established in the State (Michaud, 2002). However, there was little effect of *T. radiata* on the ACP population in Florida.

A critical finding from studies on psyllid parasitoids is that they substantially reduce populations of the pest only if the introduced parasitoid is free of its hyperparasitoids (Aubert and Quilici, 1984). To our knowledge, the only report of successful HLB mitigation through biological control was accomplished on Reunion Island, where citrus production had been essentially eliminated by HLB. Psyllid biocontrol was established by introducing two psyllid parasitoids, *Tamarixia radiata* and *T. dryi*, without their hyperparasitoids. The fact that these psyllid parasitoids were initially absent from the island was assurance that hyperparasitoids of the psyllid parasitoids also were absent. A second reason for success on Reunion Island is the ability of *T. dryi* to parasitize, and multiply on, not only *Trioza erytreae* (a vector of HLB), but also an additional psyllid, *Trioza eastopi* (not a vector of HLB), living on a very common Reunion shrub, *Litsea chinensis*. Thus, sufficient populations of *T. dryi* were maintained. Orchards on Reunion Island were successfully replanted with healthy citrus (Aubert et al., 1996). More detailed summaries of this project and other biocontrol efforts against ACP are provided by Halbert and Manjunath (2004).

Biological control, applied alone, appears to have only limited capabilities for HLB mitigation in Florida (Halbert and Manjunath, 2004). Citrus is a perennial crop and HLB has a persistent/propagative relationship with the vector. This means that the pathogen multiplies in the vector and an infected psyllid remains inoculative its entire life. Freedom from hyperparasites and abundant alternative hosts for the parasitoids are conditions that are difficult to satisfy under Florida conditions. Hence, keeping ACP populations below economic thresholds in the near term is most likely best achieved by insecticide applications. However, many of the broader spectrum insecticides are not compatible with biological control of ACP or other pest species. Increased emphasis should be placed on efforts to incorporate insecticidal practices that are compatible with biological control to ensure the long term stability of the system. Armored and soft scale, whiteflies, mealy bugs, and aphids are normally under good biocontrol in Florida and require only very limited insecticide applications. Continued efforts are likely to help maintain biocontrol agents at the highest possible levels, for management not only of psyllids but of other insect pests as well.

Under warm, humid conditions, entomophagous fungi have been observed to exert high mortality on ACP populations (Aubert, 1987). However, the most effective uses of entomopathogens like *Beauveria* spp., *Metarhizium* spp., *Verticillium vecanii* and *Paecilomyces fumosoroseus* have been in managed greenhouses with ornamental crops rather than in orchards (Osborne and Landa, 1992).

Protection against HLB by guava plants. In the Mekong delta of Vietnam, farmers have found that the presence of guava trees close to citrus trees prevents or at least retards HLB. It has recently been shown that the volatiles of guava repel ACP, and this likely explains the guava-tree effect (Noronha and Bento, 2008). While planting one or several guava trees next to each citrus tree would not seem to be a very practical solution for large orchards, use of repellents from guava volatiles to reduce ACP contact with citrus is under investigation.

REVIEW AND ASSESSMENT OF CURRENT HUANGLONGBING MANAGEMENT STRATEGIES IN FLORIDA

In the absence of curative treatments or effective biological control, preventing citrus trees from becoming infected with HLB-associated agents appears to be the only way at present to cope with HLB. The pioneering HLB researcher K. H. Lin promoted in China, in the middle of the 20th century, a three-pronged approach to combating HLB after its appearance in a region: (i) elimination of liberibacter inoculum by removal of symptomatic trees, (ii) insecticide treatments to keep psyllid vector populations as low as possible, and (iii) production of healthy citrus trees in “closed”, insect-proof nurseries for new orchards as well as for replacements of removed symptomatic trees.

In South Africa, where the less aggressive *C*Laf occurs, effective management has been achieved by the use of disease-free nursery stock, aggressive psyllid control, and removal of infected trees. Planting citrus in warmer areas that are less favorable for the disease has proven quite effective (Box 2-1). Preventative control measures have been effective for *C*Las management in some areas of China, but *C*Las is clearly more difficult to manage (Box 2-2). In areas where HLB has been more recently introduced such as Brazil, similar measures have been undertaken, but have had to be applied aggressively to achieve success (Box 2-3).

Experience with tree removal and psyllid management in Brazil’s São Paulo State allowed researchers to identify factors that significantly influence HLB mitigation. These factors are: size of the farm, age of trees, the frequency of HLB incidence in the region of the farm’s location, presence or absence of neighboring orchards without HLB-management, percentage of HLB-affected trees at first inspection, date of first inspection, number of inspections for affected trees, and the number of insecticide treatments (Belasque, Jr. et al., 2009). Similar observations have been made in Florida. Large farm size and surrounding areas well maintained and low in inoculum, as expected, reduce HLB incidence by reducing the number of incursions per unit area in the orchards.

In Florida, the same three measures are currently recommended (Brlansky et al., 2009). Production of citrus nursery trees from disease-free sources in screened enclosures has been mandatory in Florida since January 2008. The management strategy of inspection and removal of symptomatic trees and psyllid control in the field has varied in effectiveness depending on the local situation such as disease incidence in the grove, incidence in adjacent groves, and the rigor with which the program has been applied.

Survey Methods and Tree Removal

Visual Inspection

Most of the detection of HLB is done by survey crews inspecting individual trees visually for disease symptoms. In São Paulo State, and now in Florida, platforms with two or four inspectors that allow for inspection of the tops of adult trees have been found to be essential as many affected trees show symptoms first on the upper half of the canopy. Inspection by vehicle or even on foot is complicated in coastal areas where groves are planted on two-row beds with shallow ditches between beds. Visual inspection (scouting) has been very effective where highly trained crews inspect several times per year, but not otherwise. Some inspectors have an amazing ability to find HLB even when a single twig on a tree is showing symptoms. Generally, if the HLB incidence is low, inspectors will find a number of trees affected during the first three or four inspections. Then the number of symptomatic trees decreases in subsequent surveys as only more recently infected trees will be discovered. However, even in the best situations, affected trees continue to be found. In areas where incidence of symptomatic trees is very high, i.e. > 5–10 percent, tree removal and psyllid control would probably not be very effective in reducing HLB incidence.

Currently, four inspections per year are recommended (Brlansky et al., 2009). However, it has proven difficult to find HLB symptoms in spring and early summer when the new flush of growth covers the mature leaves. Thus, many growers are inspecting only two to three times a year from early fall to late winter. Where disease incidence is low and psyllid populations are in check, growers have found that this system has been effective; they have observed a decline in incidence. However, in many cases, 2 to 2.5 years of intensive effort are required before the incidence stabilizes at a low level. Many growers in areas with low HLB incidence in the central and northern production areas are not inspecting frequently enough or are using inadequately trained inspectors. In the southern production areas, where HLB incidence is high, inspections and psyllid control have been abandoned and many growers continue to harvest groves as long as they can to maximize profits. Once a grove is no longer generating a profit, growers may remove entire blocks of trees. Frequent inspections with well-trained crews are very costly and with the current low prices of fruit, growers look to minimize costs wherever possible. If the detection of HLB-infected trees is to be the first course of action even in the long term, then survey methods need to be improved.

PCR Detection

Early in the epidemic, Southern Gardens (the citrus division of US Sugar Corporation) established a diagnostic laboratory for PCR detection of HLB. Southern Gardens was one of the first large growers affected by the disease and developed the laboratory for their own use. In addition, Southern Gardens offered the service free to any grower around the state as well as for researchers and regulators. The Southern Gardens Diagnostic Laboratory (SGDL) continues to operate and is now partially funded by the University of Florida and the Florida Citrus Production Research Advisory Council. They currently use real-time PCR and have a very efficient system for processing samples and promptly notifying growers of their results. That laboratory was crucial early in the epidemic because they provided verification of samples from inspectors. It was a vital tool for training of inspectors and confirming suspected symptomatic trees. Detection of HLB-associated pathogens in asymptomatic trees by PCR also is possible (Irey et al., 2006), but due to the non-uniform distribution and slow spread of the pathogen in the citrus tree, detection is difficult. SGDL has processed over 105,000 samples since its inception and currently, processes 3,000 to 5,000 samples per month depending on the season. Other laboratories have opened and supplement the effort by the SGDL. The laboratory at the Southwest Florida Research and Education Center has processed more than 13,000 samples mostly for local growers and for research. The need for PCR testing of samples should be less essential now because inspectors can detect visual symptoms with confidence, but large numbers of samples continue to be submitted.

Tree Removal

The current recommendation is to remove symptomatic trees immediately upon detection and to spray these trees with a contact insecticide prior to removal (Rogers et al., 2010). However, prompt removal of HLB-affected trees presents a costly logistical problem for growers. Traditionally, declining trees are removed with a front-end loader with all the roots and carried to an open area to be burned. That is a costly process especially when only a few scattered trees are removed. More recently, growers usually clip the trees off at ground level and treat the stumps with herbicide to kill the roots. Research is currently being conducted to test the effectiveness of killing trees in place by applying herbicide in combination with an insecticide to kill any psyllids present. If successful, affected trees can then be removed at the grower's convenience and reduce costs for tree removal.

BOX 2-1
Huanglongbing Management in South Africa

Huanglongbing (HLB) in South Africa is associated with the heat-sensitive *Candidatus* Liberibacter africanus and the psyllid vector is *Trioza erytreae*, which is also heat sensitive. In the mid 1970s, citrus production was virtually eliminated by HLB in the major citrus-producing areas in South Africa. It was estimated that 4 million of the 11 million trees in South Africa were infected with the disease. After suffering serious losses, the South Africans adopted an HLB management program which involves three basic measures:

- Creation of the Citrus Improvement Program- production of young trees free of HLB in insect-proof nurseries
- Removal of diseased trees (instead of cutting of diseased branches) to eliminate sources of inoculum
- Control of the psyllid vector *Trioza erytreae* through the use of insecticides that are applied to the tree trunks (metamidophos, imidacloprid, and acetamiprid) and more recently, applied to the citrus roots as soil drenches

Adoption of this program and a dry climatic cycle (which was unfavorable for the vector and HLB spread) allowed for the production of citrus for export in areas where HLB was endemic, from 1990-1999. However, the end of the dry cycle in 2002, combined with the less aggressive implementation of the control measures, eventually resulted in thousands of trees becoming infected. The largest removal of orchards since the 1970s occurred in 2002. This experience illustrates the importance of holding to a control program even after the threat appears to have been reduced.

BOX 2-2
Huanglongbing Management in China

Huanglongbing (HLB) in China is associated with the heat-tolerant *Candidatus* Liberibacter asiaticus and the psyllid vector is *Diaphorina citri*, which is also heat-tolerant. Four citrus regions are affected by HLB in China; these regions are Guangdong, Guangxi, Fujian and Taiwan. Huanglongbing is less important or is absent in other regions where citrus is grown.

In Guangdong Province, citrus is grown in lowland coastal areas (where HLB incidence is high) and also in high inland areas near hillsides and low mountains, where the incidence of disease has remained low. While this phenomenon is not clearly understood, low HLB incidence has been partly attributed to the cooler temperatures in the inland areas and the geographical isolation provided by the mountains and valleys.

Current HLB management strategies in China are based on the control tactics developed by Professor K.H. Lin over 50 years ago, which remain the hallmark of HLB control worldwide. The following practices comprise the HLB management program:

- Production and maintenance of clean citrus stock in insect-free greenhouses and screenhouses
- Identification and removal of symptomatic trees
- Application of chemical insecticides to control psyllid populations
- Moving citrus production from areas where HLB incidence is high to new locations with cooler weather and are more isolated from existing citrus farms.

Hedging and Flush Control

Groves in Florida are hedged and topped with large machines to control tree size and allow passage for harvesting and other grove operations. This practice stimulates new growth flushes which are very favorable for psyllid reproduction. An insecticide application is recommended prior to hedging or topping of groves.

Although it makes sense conceptually, efforts to control flushes to achieve better control of psyllids have not been highly effective. Growth of citrus trees is highly dependent on three factors: temperature, soil moisture, and nitrogen availability. In Florida groves, a growth flush occurs in spring after temperatures increase and that flush is relatively uniform. A second flush usually occurs in June following the beginning of the summer rainy season, but that flush is often less uniform. Thereafter, trees produce growth more sporadically through late summer and into the late fall or early winter. In the warmer areas of southeast Florida, a few trees may produce new growth even in winter. Growth on young trees is more frequent and sporadic than on mature trees. Research results have shown that young trees require an aggressive psyllid control program involving systemic insecticides, usually aldicarb or imidacloprid applied to the soil. Flushes can be managed to some degree by water and fertilizer applications, but to achieve this during specific periods in order to control psyllids is not currently possible.

BOX 2-3
Huanglongbing Management in São Paulo State, Brazil

Huanglongbing (HLB) was first detected in the São Paulo State (SPS) of Brazil in 2004. The disease is associated with *Candidatus* Liberibacter asiaticus and *Candidatus* Liberibacter americanus and the insect vector is *Diaphorina citri*. As of April 2008, it was estimated that there were 1.15 million trees with symptoms of HLB and at least 3 million trees that have been removed and destroyed (Belasque, Jr. et al., 2009). The HLB Management Program in SPS consists of these methods:

- Inspections to identify symptomatic trees (ground inspection, inspection using tractor-pulled platforms or inspectors on horses)
- Immediate elimination of symptomatic trees
- Application of insecticides against the psyllid vector *Diaphorina citri*
- Use of citrus produced in insect-proof nurseries when replanting

A 2008 study of 20 farms that had employed the HLB management practices for four years indicated that very good HLB control (i.e. low percentage of HLB-affected trees) was achieved in farms that:

- were located in municipalities with low HLB incidence
- had older trees (with fewer growth flushes and less attractive to psyllids)
- were located at least 3 km away from orchards that do not control HLB
- had started the HLB management program early on (immediately after HLB was detected in the farm)

In farms that were located in municipalities that had high HLB incidence and were adjacent to orchards that do not control HLB, good to fair level of HLB control was achieved through a high number of inspections and insecticide applications. In general, poor to very poor level of HLB control was observed in farms that had all or some of these characteristics:

- located in municipalities with high HLB incidence
- bordered by orchards that do not control HLB
- had young trees
- had a fewer number of inspections and insecticide applications
- started the HLB management program late

Insecticidal Control of Psyllids

Many insecticides that are effective against psyllids are currently registered for use on Florida citrus. The characteristics of these products, their modes of action and their activity against other pests are presented in the 2010 Florida Citrus Pest Management Guide (Rogers and Dewdney, 2010). The classification of the mode of action of each of these materials with respect to resistance management is also available in the Florida Citrus Pest Management Guide (http://edis.ifas.ufl.edu/cg026) and from the Insecticide Resistance Action Committee (http://www.irac-online.org/) of the Entomological Society of America.

To keep psyllid populations in check, insecticides must be applied frequently. This raises environmental concerns, reduces the effectiveness of biological control, and increases the potential for the psyllid to develop resistance to different classes of compounds.

Young Orchard Trees

Imidacloprid, a systemic compound, is applied as a soil drench for psyllid control on trees less than 2 m in height. A single application in the spring is effective for psyllid control in young trees for several months. In some cases, applications at half the maximum annual rate can be applied twice a year for season long control. Subsequent applications of foliar insecticides often are required in the summer and fall to attain good psyllid control. In an alternative recommended control practice, the observation of relatively low psyllid populations during mid-summer and winter (due to temperature extremes that are unfavorable to ACP) suggests value in spraying at these times. A winter spray may greatly reduce ACP populations in the spring. While imidacloprid is also effective for large trees, the registered rates are only sufficient for control of insect pests on small trees. Use of higher rates to control ACP in large trees would not be in accordance with the label and would be excessively expensive (M. Rogers, Citrus Research and Education Center, Lake Alfred, FL, personal communication).

Mature Orchard Trees

Soil application of aldicarb is effective for psyllid control on mature trees (trees greater than 2 m in height or 5 years of age). Recommedations for aldicarb use on large trees are based on the numerous UF/IFAS field trials that have been conducted with aldicarb for psyllid control since 2005 (M. Rogers, Citrus Research and Education Center, Lake Alfred, FL, personal communication). Applications in Florida citrus are limited to the November–April period to avoid leaching of the product into ground water during summer rains. No applications are permitted within 1000 ft of a drinking water well. Often, growers apply aldicarb to mature groves to reduce the numbers of overwintering adult psyllids and avoid build-up on the spring flush. Soil application in winter avoids the need to apply foliar insecticides during the bloom period when bees are active. However, aldicarb applications may not be completely effective if they are not followed by sufficient rainfall to move the material to the roots.

Many foliar insecticides are effective for killing psyllids including: carbaryl, chlorpyrifos, dimethoate, fenpropathrin, imidacloprid, malathion, petroleum oil, phosmet, spinetoram, spirotetramat, and zeta-cypermethrin. The registration for spirotetramat has been recently at least temporarily vacated (CCQC, 2010). Some are less damaging to populations of beneficial insects than others and alternating products could help avoid resistance problems. Products also differ in their effects on other citrus pests, and choice of product may also be affected by the need for control of other pest problems. Recent research indicates that the incidence of the HLB bacterium in psyllids is highest in the spring and in the late fall. Thus, applying pesticides during these times is most effective and avoiding summer sprays may allow recovery of biological control agents for psyllids and other pests. Regarding bearing trees, an interesting development concerns the use of broad-spectrum foliar sprays during the winter season when the trees are dormant and produce no flush. These sprays target adult, overwintering psyllids. Similar to the situation with young trees, by eliminating these adults, psyllid populations will be greatly reduced on the following spring flushes.

Application Methods

Traditionally, foliar applications to Florida citrus have been made with airblast sprayers using spray volumes of 100 to 250 gal/acre. Some sprays are made with the Curtec sprayer utilizing 30 gal/acre, but they are not effective for some pests and diseases and not all products are registered for low-volume applications. Reducing spray volume and increasing the velocity at which applications are made greatly reduce the cost of insecticide applications. Recent research indicates that low volume and aerial applications of insecticides are effective for psyllid control. Most of the above products are registered for low-volume and aerial application butonly malathion is registered for aerial application at ultralow volume. It is important to control psyllids on an area-wide basis so that psyllid populations cannot build up between applications and move between groves so readily.

SIGNIFICANT CITRUS DISEASES AND INSECT PESTS OTHER THAN HUANGLONGBING

In addition to HLB, many other diseases can produce serious problems with citrus production. Systemic (graft-transmissible) diseases are often more serious in the sense that they may kill or completely debilitate trees and reduce production, but many foliar fungal and bacterial diseases may also reduce yield as well as blemish fruit. Many diseases are already widespread and most citrus areas have to deal with them. All of the important diseases are described in some general references such as the Compendium of Citrus Diseases (Timmer et al., 2000) and various other books and book chapters (Roistacher, 1991; Timmer and Duncan, 1999; Timmer et al., 2003; Timmer et al., 2004) and on the website of the International Organization of Citrus Virologists (http://www.ivia.es/iocv/). Many insect and mite pests are also described in publications such as the Florida Citrus Pest Management Guide (http://www.crec.ifas.ufl.edu/extension/pest/index.htm) and other local publications in different citrus areas. Many diseases and insect pests are not uniformly distributed, and those that pose threats if introduced into new areas are given in Appendices D and E respectively. Several vector species are listed which are minor pests alone but become important once the pathogen they transmit is introduced.

ECONOMICS OF CITRUS PRODUCTION IN THE PRESENCE OF HLB

Effect of HLB on Yields and Costs of Production

HLB is a devastating disease and even if it can be managed, costs of production will increase dramatically. The disease is controlled by a combination of methods: 1) the use of disease-free nursery stock; 2) survey, detection, and removal of symptomatic trees; and 3) control of the psyllid vector. The costs to citrus production include:

- decline in yield and fruit quality of affected trees.
- survey for detection of infected trees.
- removal of the HLB-affected trees.
- soil-applied and foliar insecticides for psyllid control.
- increased cost of production of disease-free nursery trees.
- cost of replacement tree, care of replant, loss of production until replant becomes actively bearing (3 years).

Yield Loss

The effects of yield losses and the rapidity with which they occur are uncertain since there is little experience to date in Brazil or Florida. However, any young trees that become infected will never produce a commercial crop of fruit. The rate of decline of older trees depends largely on the number of infections that occur. If a tree has only a single infection, it is likely that the tree will remain productive for several years. However, if multiple infections occur in a single year to cause a systemically wider disease within a tree, its remaining productive life may be as short as two years. Even under the best of circumstances, grove life is likely to be shorter than it has been in the past. It is very difficult to quantify the cost of declines in yield and loss of entire groves that are likely to occur in Florida over the next few years.

Direct Control Costs

Nursery Trees

The January 2008 requirement for production of citrus nursery stock in screened enclosures has doubled the cost of nursery trees from $4–5 to $8–10 per tree. Prices have begun to decline recently as supply is beginning to catch up with demand, but nursery tree prices remain high and a significant cost of replanting citrus groves. Those costs may be especially significant if growers choose higher density plantings anticipating losses of trees to HLB.

Inspections and Tree Removal

At the present time, four inspections per year are recommended (Brlansky et al., 2009). Each inspection is estimated to cost about $25–30/acre or $100–120 per acre per year (Morris et al., 2008). Tree removal costs vary considerably depending on the number of trees to be removed and the method used. Muraro (2008b) estimated the cost of tree removal at $34/acre/year, assuming that six trees per acre would need to be removed each year. The average grove in Florida has about 145 trees per acre and thus, six trees would represent about a 4 percent loss per year. That cost could be considerably higher in many situations. Tree removal costs are expected to be high initially for removal of already-infected trees and then decline and stabilize if the disease is brought under control.

Morris et al. (2008) analyzed two approaches to dealing with HLB: 1) remove affected trees and replant with young trees immediately and 2) leave affected trees and remove the entire block when production was no longer economically viable. They concluded that there was little difference economically between the two scenarios. The most desirable approach depends on the situation. Since young trees are very attractive to psyllids and readily infected by HLB, replanting of individual trees is probably only reasonable if HLB incidence in the area and the likelihood of infection are very low. Young trees can be effectively protected by soil applications of imidacloprid, but application costs are very high (about $0.50/tree/year) and higher where there are only a few trees per acre to be treated. In one large planting in southwest Florida, nursery trees are grown to 3 years old in pots in a protected area and treated with imidacloprid at maximum rates and frequencies prior to planting in the field to avoid numerous trips through the grove to apply the insecticide. From an operational point of view, it would probably be simpler to wait until the grove was no longer economically productive and then remove the entire block. However, that leaves large numbers of HLB-infected trees in the planting that serve as sources of inoculum for healthy trees. Thus, it is probably advisable and advantageous to remove infected trees promptly and then remove the entire block when too few trees remain to be economically viable.

Insecticide Applications

Morris et al. (2008) estimated that for the average grove of mature trees, a soil application of aldicarb and three applications of foliar insecticides would be needed for psyllid control in the presence of HLB in addition to normal sprays for control of other pests and diseases. Such a program would be similar to the possible control approaches that are recommended by the University of Florida (Rogers et al., 2010). The cost for those products and their application was estimated to be $288 per acre per year (Morris et al., 2008). Costs can vary considerably depending on the situation. They could be reduced by using a dormant foliar insecticide application rather than aldicarb in the winter, but could be substantially higher if more applications are needed to control psyllid populations.

Effect on Returns and Profits

The costs and returns on Florida citrus vary greatly depending on the variety of citrus grown, its destination (fresh or processing), and the yields attained. Citrus fruit is often sold on the tree and the purchaser is responsible for harvesting and transport of the fruit. Growers are paid for processing fruit on a pounds-solids basis (the sugar content of the fruit) as assessed upon delivery. Yields are usually expressed as the number of 90-lb boxes per acre and the average sugar content in Florida is about 6.5 lb per 90-lb box but varies considerably depending on the variety, rootstock, soil conditions, and management practices. Processing fruit is harvested by hand or occasionally mechanically and transported in bulk in open trucks to the processing plant. Muraro (2008b) estimates the cost of production for Valencia oranges in Central Florida at $1657 per acre including management and interest on investment for a grove assuming no costs for HLB or canker control. Harvesting, delivery and assessment costs are estimated to be $1226 per acre for a total delivered-in cost of $2883 per acre for an average grove. The break-even price for a grove with no HLB or canker would range from $1.19 to $0.80 per pound solids if yields ranged from 300 to 600 90-lb boxes of fruit per acre assuming no resetting of the trees removed. In the presence of HLB and canker, the break-even price would be $1.38 to $0.89 in groves with yields ranging from 300- to 600-box yields.

Fresh fruit is harvested in 10-box tubs (~ 900 lb total) and growers are paid on a per box basis. With the same assumptions as above, the total delivered-in cost for production of white grapefruit in the Indian River area would be $3,195 per acre without canker or HLB and $3,600 in the presence of both (Muraro, 2008a). Break-even prices would range from $8.37 to $5.82 per box for yields ranging from 350 to 650 boxes per acre in the absence of HLB and canker and $10.03 to $6.71 in the presence of both diseases.

Morris et al. (2008) conclude that production of citrus for processing can be profitable in the presence of HLB, but production costs would increase by about 41 percent. That assumes that tree loss rates and costs for inspections and psyllid sprays are in the ranges indicated above and the value of orange juice is $1.25 to $1.50 per pound solid. In the past season (2008–2009), juice prices ranged from $0.75 to $0.85 for early and midseason varieties and $0.80 to $1.25 per pound solid for Valencia oranges (http://www.flcitrusmutual.com/market_info/citrusprices.aspx). Early and mid-season oranges generally have a lower price than Valencias, but some of the differential is due to increasing prices late in the season. Average delivered-in prices for Valencias have ranged from a low of $0.77 per pound solid in 2003–2004 to a high of $2.23 in 2006–2007 (Morris et al., 2009). Processing grapefruit prices are very depressed due to large inventories and ranged from only $0.25 to 0.80 per pound solid, but good quality fresh grapefruit still demands a high price and is a profitable crop. Morris et al. (2008) suggested that higher planting densities could be used to partially offset the increase in production costs per acre.

Orange Juice Production, Consumption, and the Competitiveness of the Florida Industry

Most of the orange juice consumed in the world is produced by Brazil (50 percent) and Florida (35 percent). Total world consumption of orange juice is about 3.3 billion gallons of single-strength equivalents (SSE) and the United States and the European Union consume about 1.3 billon SSEs each (Spreen et al., 2008). Florida supplies most of the orange juice consumed in the United States (77 percent) (USDA-NASS, 2009) and Brazil supplies most of the juice consumed in Europe and elsewhere in the world or about 1.25 billion gallons (USDA-FAS, 2008). The per capita consumption in the United States declined from 5.8 to 3.9 gallons per year from 1997–1998 to 2007–2008 and continues to decline and inventories are still high (Morris et al., 2009). That may be attributable to popular low-carbohydrate diets, the availability of other juices and beverages, and increased cost or other factors. China produces some juice currently and has the potential to become a major player in the juice market as their production has increased significantly in recent years.

There are several factors that affect the ability of Florida citrus growers to compete for the market for citrus juice. There is currently a tariff on juice imported from Brazil of $0.29 per pound solids which is equivalent to about $0.17 per gallon of single strength juice. Elimination of the tariff for Brazil is estimated to reduce the price of juice in the United States by $0.22 per gallon (Brown et al., 2004). Elimination of all tariffs in the United States, Europe, and Japan is estimated to reduce the price of juice in the United States by $0.13 per gallon. Juice imported from Mexico and Central America is not subject to tariffs. Thus, if tariffs are eliminated, production in Florida would have to become more efficient to compete.

Production costs do not differ greatly between São Paulo and Florida. Generally, labor costs are lower in São Paulo, but costs for machinery, pesticides, and fertilizers are higher in Brazil than in the United States. However, the cost of harvesting, which involves a great deal of labor, is significantly lower in Brazil than in Florida and thus, the total cost for production of juice is greater in the United States. HLB will increase costs of production in São Paulo as well as in Florida. However, survey costs for HLB detection are estimated to be only $4 per acre in São Paulo compared to about $25–30 per acre in Florida. Other costs of control would be similar in the two locations. Other potential producers of orange juice either already have HLB or face the prospect of having it in the near future. Thus, it is unlikely that any area would have a competitive advantage over São Paulo or Florida long term because of the absence of the disease. Thus, São Paulo would be the main competitor for Florida in the foreseeable future. At current prices, processing orange production is not highly profitable for most growers. However, large vertically integrated companies that grow, harvest, process, and sell their own products are in a much better position to deal with price fluctuations. Profitability of citrus in Brazil is also affected by the value of the dollar since most of their returns are received in US $ and most of their expenses are paid in Brazilian reis. Thus, a high value for the dollar is beneficial to Brazilian producers. It is likely that the large Brazilian production companies, Citrovita, Citrosuco, and Cutrale, will control a significant portion of the production in both São Paulo and Florida in the future. In both locations, large companies with extensive land holdings will have an advantage over small growers who cannot control the actions of their neighbors. Small growers probably can only survive by forming cooperative arrangements with their neighbors for HLB control.

There is adequate land available for citrus production in Florida despite considerable urban growth and development. Much of the land devoted to citrus is not suitable for other crops. Large acreages are available in São Paulo and citrus competes primarily with sugarcane (Spreen et al., 2008). Due to considerable utilization of sugarcane for ethanol production, prices of sugar have increased in recent years. Many of the groves severely affected by HLB have been converted to sugar production. Competitiveness in Florida may depend on reducing harvesting costs. Mechanical harvesting is already used to some extent and, if an abscission chemical can be registered, a large portion of the processing oranges could be harvested in that fashion. Thus, if HLB control can be achieved, production of processing oranges should continue in the foreseeable future, although the acreage is likely to be lower than in the past.

FEDERAL, STATE, AND LOCAL REGULATORY AGENCIES AND INDUSTRY ORGANIZATIONS AND THEIR IMPACT ON THE CITRUS INDUSTRY

There remains an urgent need to maintain speed and focus of research aimed at finding an effective solution for HLB. New technologies intended to combat HLB may include new early detection technology, new chemicals, transgenic plants and psyllid control by means of biological tools. A strong public-private partnership and commitment will be required to move any new technologies forward through regulatory agencies, to public acceptance and, ultimately, to commercial implementation. As is indicated below, many organizations are concerned with research related to citrus production and processing. This diversity of agencies presents both an advantage, by allowing many points of view to be developed, and a challenge, because of the need to channel efforts to combat HLB.

In order to develop a picture of the Florida citrus industry's many moving parts, we provide Table 2-4 which arranges the various agencies with influence over citrus production, processing and marketing or advocacy according to their status (university, government, non-government) and the type of benefit provided. Any attempt to pigeon-hole the many private and public agencies whose actions impinge on citrus production, processing and marketing will inevitably be inexact and subject to challenge. Nevertheless, we find the Table 2-4 distribution of agencies to be useful, even though some are of hybrid type and some have functions that are overlapping. Appendix G presents short mission statements for each of the agencies.

The concern and sense of urgency over the HLB situation in the Florida citrus industry prompted the awarding of unprecedented amounts of funding for research aimed at developing short-, intermediate- and long-term approaches to mitigating the disease. Some in the industry and the research community are advocating a “Manhattan Project” type of dedicated and integrated research program on HLB mitigation. There is no road map for such an unprecedented undertaking in agricultural research, but whether a more or less focused course is taken for research and development on citrus, there will be possible organizational overlap and redundant responsibilities and funding mechanisms. Some industry leaders have expressed apprehension about the apparent fragmentation of research effort in the past and hope for a more integrated approach in the future. There is general recognition that investments into an insightful, focused, sustained and stable research funding program provide the best hope for survival of the citrus industry. Moreover, coordination of efforts in controlling HLB likely will lay the groundwork for dealing with future challenges to the profitability of the citrus industry.

RELATIONSHIPS OF FEDERAL, STATE, AND INDUSTRY ENTITIES IN FLORIDA AND HOW THEY ADDRESS CITRUS DISEASES

Intra-Industry Relations

The citrus industry of Florida is not a unified force with similar interests. Rather it is a composite of many interests. The two large processing corporations, Tropicana and Minute Maid, do not grow citrus, but instead buy the fruit on the open market or via contracts with growers in addition to purchasing juice from Brazil and other countries. They have generally been opposed to a box tax for marketing purposes and prefer to advertise their own products rather than pay for generic advertising for Florida orange juice and other products. Most growers prefer generic advertising to compete with Brazilian and other producers of juice and fresh fruit. The growers of processing and fresh fruit also have different interests. While production of processed oranges represents the majority of the acreage in Florida, both are economically important because fresh fruit provides far greater income per acre. The main fresh fruit product in Florida is grapefruit, but some tangerines and navel oranges are also produced. The Indian River Citrus League was formed primarily to support the interests of growers of “Indian River Grapefruit”. Most of the industry-wide committees have members representing the processing and the fresh industries and include representatives from various areas of the state.

Government Organizations

The responsibility of the USDA-ARS is research, that of the University of Florida (UF) is research, extension, and education, and that of DPI is regulatory, with each of those entities restricting their efforts to those ascribed areas for the most part. The relationship between the principal government organizations dealing with HLB have generally been cordial although occasional conflicts have arisen where the boundaries of these areas blur. There has been some competition and infighting among them, as well as among research and extension stations within

UF. This competition has sometimes been detrimental to the development of a consistent policy and message to growers on dealing with HLB. However, most of the competition has been among agency and department heads for funds and influence, and probably has not greatly affected cooperation among individual investigators. Cooperation among investigators from the different agencies has often been very good. Since most of the agencies are not organized along disciplinary lines, there are few boundaries to research among the various areas of investigation. Entomologists, pathologists, and horticulturists cooperate readily and as needed to achieve specific goals.

Table 2-4 Government, University and Non-Government Agencies with Responsibilities Related to Citrus Production and Marketing[a, b, c]

	University	Federal, state or local government	Non-government
Citrus research	UF-IFAS	ARS (USDA) FCPRAC FDOC	CRDF FCIRCC
Citrus protection	UF-IFAS-Extension	APHIS (USDA) DPI (FDACS)	US Sugar Commercial pesticide suppliers
Citrus production and processing		CAC (US) CHRP (APHIS/DPI) County governments DEP (FDACS) Water management districts	FCP FCPA FCPMA FFSP FGFSA FNGLA NVDMC Regional grower associations
Citrus promotion and marketing		FDOC	FCM

[a]Several agencies are multifunctional. Placement in the table indicates the agency's main function or functions

[b]Acronym List:

APHIS: Animal and Plant Health Inspection Service (USDA)
ARS: Agricultural Research Service (USDA)
CAC: Citrus Administrative Committee
CHRP: Citrus Health Response Program
CRDF: Citrus Research and Development Foundation
DPI: Division of Plant Industry (FDACS)
FCIRCC: Florida Citrus Industry Research Coordinating Council
FCM: Florida Citrus Mutual

FCP: Florida Citrus Packers
FCPA: Florida Citrus Processors Association
FCPMA: Florida Citrus Production Managers Association
FCPRAC: Florida Citrus Production Research Advisory council
FDA: US Food and Drug Administration
FDACS: Florida Department of Agriculture and Consumer Services
FDOC: Florida Department of Citrus/Florida Citrus Commission
FFSP: Florida Foundation Seed Producers, Inc.
FGFSA: Florida Gift Fruit Shippers Association
FNGLA: Florida Nursery, Growers and Landscape Association
NVDMC: New Varieties Development and Management Corporation
UF-IFAS: University of Florida-Institute of Food and Agricultural Sciences
USDA: US Department of Agriculture

[c] Several agencies of the US federal government have authority to regulate aspects of citrus production and processing but are not specific to the citrus industry. These are the following:

DOL: US.Department of Labor
EPA: US Environmental Protection Agency
FDEP: Florida Department of Environmental Protection
ICE: US Immigration and Customs Enforcement
OSHA: US Occupational Safety and Health Administration

Government-Industry Relationships

Relations between the USDA-ARS and industry have been cordial. USDA research efforts have contributed significantly to the understanding of HLB, and USDA-organized research conferences, to which growers have been invited, have been valuable. The University of Florida (UF) has the primary responsibility for extension and providing information to growers in addition to its leadership role in research and education. A serious effort has been made by UF to inform growers about HLB and the best management practices. HLB has been the primary topic for every grower meeting for the last few years. Speakers from the USDA and DPI are often on the program and cooperation has generally been good among the agencies in providing current information to growers. Local meetings have been organized primarily by DPI, UF and Florida Citrus Mutual to bring the latest information on canker and HLB to growers in each area of the state. Attendance at such meetings has been excellent, and growers have had numerous opportunities to become current on the HLB and canker situation and the recommended practices. However, individual extension agents hold differing views on the best management practices for HLB and have not always followed the consensus approach of prompt tree removal and intense psyllid control. This situation has left some growers confused about what recommendations to follow. Within UF, the Citrus Research and Education Center is the primary agency for research and education for citrus in the state, but the UF Southwest Florida Research and. Education Center and UF Indian River Research and Education Center, as well as faculty in various departments on the main campus, contribute significantly to the effort. To some extent, the experiment stations in southwest Florida and the Indian River reflect the interests of the industries in their areas, and industry jealousies and competition carry over into research and extension.

The FDACS-DPI and USDA-APHIS have the primary responsibility for regulatory matters. Those agencies were responsible for all of the citrus canker eradication programs (see Appendix H). In the 1980s, an eradication program was conducted for a disease, thought to be canker, which turned out to be citrus bacterial spot, another bacterial disease of relatively minor importance. However, citrus canker was discovered as well and infected trees were eradicated. Many nurseries were destroyed, many probably unnecessarily, during that time and many nurserymen still resent actions taken by DPI during that period. The canker eradication program conducted during the late 1990s and early 2000s was generally supported by growers and most nurserymen. Growers and nurserymen were mostly well-compensated for the losses during that program. However, at the end of the program, growers were losing confidence, felt that eradication was continued long after it was apparent that it could not succeed, and compensation for losses was greatly delayed. Thus, there is some resentment among growers toward DPI and USDA-APHIS due to those programs. Many lawsuits are still pending between DPI and a few nurserymen and a homeowners group, who do not believe that they were adequately compensated.

The potential impact of the discovery of the ACPin Florida in 1998 was ignored because of the focus on the Citrus Canker Eradication Program. The disease was not known in Florida at this time, and the ACP was not causing much economic loss. When HLB was detected in 2005, DPI chose not to conduct an eradication program or even an inoculum suppression program for the disease. This decision was reached primarily because HLB was already widespread at first detection but also because of reticence by DPI about getting involved in another widespread and problematic suppression program even though HLB was listed as a select agent (SA) under Agricultural Bioterrorism Protection Act of 2002 (CFR Part 331). The SA designation limited research on HLB to a few registered quarantine/diagnostic laboratories in the US and was a serious impediment to research progress. HLB was subsequently removed from the SA list in late 2008.

The FCPRAC has served to focus research efforts and has been forced to decide on the priorities for investigation that met the needs of all segments of the industry. The Council has greatly improved contacts between industry and the research community and has furthered mutual understanding. Since FCPRAC has provided much of the funding for citrus research at UF and the USDA in Fort. Pierce, they have greatly influenced research priorities and directions. Initially, they funded mostly research to fulfil immediate needs, but with time have seen the need for supporting more basic, long-term work. Currently, most of the funds have been directed toward HLB with small amounts dedicated to canker research.

The need to develop regulations that met the needs of the entire industry and did not excessively burden any segment has brought together many diverse interests. The Citrus Health Response Program was initiated by the USDA-APHIS and DPI with considerable input from the USDA-ARS and UF and industry groups in an attempt to provide some regulations that would benefit the entire industry. Many aspects of those regulations were onerous especially for the nursery industry and there were many hard-fought battles waged in developing a satisfactory system. However, the current system of production of all citrus trees in screened enclosures has benefited everyone in the industry.

The incursion of HLB has brought the industry and government agencies together as never before, and cooperation among all agencies has generally been good, if not always effective. Since the detection of ACP in Florida in 1998 and HLB in 2005, the various agencies and entities have been involved in activities geared towards finding ways to manage the vector and the disease (see Appendix H). Recently developed organizations such as Florida Citrus Industry Research Coordinating Council and the Citrus Research and Development Foundation represent significant attempts by the industry to coordinate all efforts focused on HLB.

The array of organizations (federal, state, university, local, or grower or producer) which impinges on citrus production and processing is large and diverse. Table 4-1 in Chapter 4 presents, in brief, five recommendations for changes in organizations and organizational activities that we believe will advance HLB mitigation. Table 4-1 is followed by notes on the five recommendations.

3

Citrus Greening Research and Development and Industry Preparedness

This chapter is concerned with research results from recently completed and ongoing research projects and their potential contributions to huanglongbing (HLB) mitigation. We begin with a discussion of research on the presumed causal agent of HLB, *Candidatus* Liberibacter asiaticus (*C*Las), and then turn to an analysis of research that is related to and supportive of current HLB mitigation practices, principally identifying and removing HLB-affected trees and reducing Asian citrus psyllid (ACP) populations in orchards. Since it is generally agreed that citrus varieties resistant to *C*Las or to ACP and preferably to both would provide the most sustainable HLB mitigation tool, we summarize research on citrus breeding, genetics, transgenics, including the potential of *Citrus tristeza virus* (CTV)-derived vectors for introduction of resistance traits into citrus. Possible contributions of new citrus cultural practices and research model systems to HLB mitigation are considered. Finally, we summarize the history of funding support for pest management and for citrus research and development generally and the importance of establishing communication channels for citrus researchers. An inventory of HLB research projects that are ongoing can be found in Appendix J and a list of HLB research milestones from 1956–January 2009 is provided in Appendix K.

*C*LAS GENOMICS AND CULTURE

The demonstration by researcher H. K. Lin in China in 1956 that the HLB agent was transmissible by graft inoculation established the infectious nature of this pathogen. As is described in Chapter 2, by 1970 electron microscopy had revealed bacterial cells with cell walls to be residing in the phloem tissue of citrus showing HLB symptoms. By 1974, the bacteria were shown to be Gram-negative. This bacterium, the only viable candidate as the causative agent of HLB in Florida, is designated *C*Las. See Chapter 2 for a discussion of the currently known HLB-associated agents and other information on *C*Las.

*C*Las Genomics

The complete genome sequence of *C*Las has been obtained by using DNA extracted from a single psyllid carrying a high titer of *C*Las (Duan et al., 2009). Metagenomic analysis of DNA from phloem of *C*Las-infected citrus revealed more than one *C*Las genome per phloem cell and only trace representation of any other bacterial cell (Tyler et al., 2009). Although the analysis was not capable of revealing viruses, the result suggests very strongly that *C*Las alone is the causal agent of HLB in Florida. Recent work by Wulff et al. (2009) and Duan et al. (2009) have shown that the genomes of both *C*lam and *C*Las appear to be circular with 3 ribosomal ribonucleic acid (rRNA) operons and similar size. Analysis of the *C*las genome has revealed many other properties of the organism, particularly the absence of pathogenicity systems involving toxins, enzymes or specialized secretion systems. The absence of such specialized secretion systems (referred to as Type III secretion systems or T3SS), which are common in Gram-negative bacteria that are pathogenic to humans, animals, insects, and plants, had led to speculation that other mechanisms of pathogenicity may be involved (Bové and Garnier, 2003). The gene for a bacteriophage DNA polymerase, discovered in 1993, has now been shown to be part of a bacteriophage DNA genome associated with HLB on citrus (Gabriel and Zhang, 2009). These results raise the question of a possible role of liberibacter phages in HLB. Sequencing also has revealed genetic diversity among *C*Las isolates collected in Southeast Asia (Tomimura et al., 2009).

Obtaining the *C*Las genome sequence has added to our knowledge of HLB, but how this information can contribute to HLB mitigation remains to be demonstrated. The genomes of several other bacterial plant pathogens have been sequenced. These include the genomes of *Xylella fastidiosa*, causal organism of citrus variegated chlorosis (and Pierce's disease of grapevine), *Xanthomonas axonopodis pv. citri*, causal organism of citrus canker, and *Spiroplasma citri*, causal agent of citrus stubborn disease. These bacteria have been cultured on synthetic media. The genomes of several phytoplasmas, which have not been cultured, have also been completed. In each case new candidate pathogenicity genes have been discovered and in some cases those genes have been functionally confirmed to contribute to pathogenicity. An interesting example is the onion yellows phytoplasma. Its genome sequence revealed that it possesses specific genes that allow it to send protein molecules out of the sieve tubes to carry out functions beneficial to the phytoplasma. This protein, called Tengu, is believed to inhibit an auxin-related pathway which affects plant development (Hoshi et al., 2009).

*C*Las Culture

When HLB was first detected in Florida in 2005, the three citrus liberibacters, *C*Las, *Candidatus* Liberibacter africanus (*C*Laf), and *Candidatus* Liberibacter americanus (*C*lam), were known, but no evidence indicates any agent for HLB in Florida other than *C*Las (Tyler et al., 2009). In 2008, co-cultivation of the Asian HLB-associated bacterium (*C*Las) with Gram-positive actinobacteria was reported by a research group in Florida, but a pure culture of *C*Las could not be obtained (Davis et al., 2008). In 2009, a report on cultivation of all three HLB-associated Liberibacters and fulfillment of Koch's postulates was published by Sechler et al. (2009). However, neither the Sechler et al. (2009) result or previous reports of successful axenic culture of Liberibacters (Garnett, 1985; Whitlock and Chippindall, 1993) have been repeated in other laboratories.

RESEARCH SUPPORTING CURRENT HUANGLONGBING MITIGATION PRACTICE: REMOVING HUANGLONGBING-AFFECTED TREES

A major problem in managing HLB is the long latent period between inoculation of *C*Las by ACP and the appearance of symptoms visible to scouts or the accumulation of *C*Las in a manner conducive to its ready detection by polymerase chain reaction (PCR). Firstly, the long latent period affects the thinking of growers who embark on a program of infected tree removal because, in the first 2 to 3 years of the program, the fraction of trees that is found to be HLB infected may increase even though the reservoir of inoculum actually is decreasing, i.e., the program is successful but appears to be not successful. Secondly, *C*Las-infected trees may may remain in the grove for months or years without apparent symptoms but with the ability to serve as a source for acquisition of *C*Las by ACP. At present, there is no practical method for detecting infected trees before they show visual symptoms. The problem is accentuated by the uneven distribution of *C*Las in the tree, which manifests itself in sectored development of symptoms, with some branches remaining asymptomatic. If infected but asymptomatic trees can be rogued, the result may be far more effective at *C*Las inoculum reduction than is possible with current scouting practice, if for no other reason than a reduction of months in the time of interaction between infected tree and psyllid. Also, the extent of *C*Las spread within the tree can be expected to be less for an asymptomatic tree than a symptomatic tree.

Irey et al. (2006) tested trees for *C*Las by PCR by using plots of about 190 trees. Leaf sampling was from the two most recent flushes and from 3 of the 4 sides of each tree, but was otherwise undirected. DNA was recovered from petioles and midribs. PCR detected about 60 percent additional HLB-infected trees beyond those detected by visual assessment alone (6.5 percent of the trees in the plots were visually HLB positive; 4.1 percent were PCR positive but visually negative). Given the difficulties of detecting infected trees by PCR analysis, the above results suggest that for every symptomatic tree in an orchard there will be at least one infected asymptomatic tree. Among the separate plots, the numbers of PCR-identified infected trees was well correlated with the total number of infected trees, $R^2 = 0.89$, suggesting a natural progression of infected but asymptomatic trees to symptomatic trees. Although the average *C*Las titer in asymptomatic trees was significantly less than the titer in symptomatic trees (M. Irey, United States Sugar Corp., Clewiston, FL, personal communication), some asymptomatic trees showed a high titer, reinforcing the importance of identifying and removing infected but asymptomatic trees. More than 80 percent of the PCR-positive trees were within 25 m of a symptomatic tree (Irey et al., 2006), indicating significant secondary, short-distance spread (Gottwald et al., 2007) but also long-distance spread not revealed by symptom development at the time of sample taking. Presumably, the distant infected but asymptomatic trees will become foci for future local HLB spread. These observations suggest that:(i) the pathogen is readily spread by the vector to adjacent or nearby trees; and (ii) spread over longer distances also occurs, possibly caused by dispersing vectors. Alternatively, dispersal over long distances could result from farming practices. Pre-symptomatic trees have been reported to be sources for *C*Las acquisition by ACP, but with reduced efficiency compared to acquisition from symptomatic plants (Coletta-Filho et al., 2009).

Sweet orange maintained in a greenhouse developed symptoms at about 90 days after being graft-inoculated with *C*Las-infected tissue. However, *C*Las was detected in extracts of leaf midribs at 30 days after inoculation. The *C*Las DNA accumulation was about 50,000-fold greater at 90 days than at 30 days (Li et al., 2006; Alvarez et al., 2007), suggesting that at least some *C*Las-infected but asymptomatic orchard trees will have low *C*Las titers and a significantly diminished ability to serve as HLB sources compared to symptomatic trees.

Detecting a *C*Las-infected tree by analysis for *C*Las is the obvious but not necessarily the most efficient approach to discovering infected trees. Perhaps the asymptomatic but *C*Las-infected tree is altered sufficiently, even in parts of the tree not yet infected, to develop a specific altered-state signal, a **biomarker**, that would telegraph the presence of infecting *C*Las. A *C*Las-infection-specific, or even general infection-specific, biomarker may be based on changes in tree volatiles production, optical properties, or chemical properties, including accumulation or loss of specific messenger RNAs (mRNAs) (see section on the citrus transcriptome, below), proteins or metabolites. These changes may be exploited as disease-specific biomarkers either directly or may be used to predict the identities of biomarkers after intensive bioinformatics analysis that can document changes in metabolic pathways and anticipate alterations in the concentrations of specific small, including volatile, molecules. Even a biomarker that was not activated by asymptomatic infection but would efficiently reflect a symptomatic infection could be of value by allowing scouting to become partially automated.

Some plants have been reported to release biologically active volatile organic compounds (VOCs) in response to infection by a specific pathogen, including fungi, bacteria and viruses (Huang et al., 2005; Cardoza and Tumlinson, 2006; Medina-Ortega et al., 2009; Werner et al., 2009). Profiling of volatile metabolites has been used to discriminate among pathogens infecting apples (Vikram et al., 2004). Production of a new, citrus canker-specific volatile by infected and symptomatic grapefruit leaves has been reported (Zhang and Hartung, 2005). Preliminary results suggest that *C*Las-infected and uninfected citrus could be distinguished based on VOCs analysis (Dandekar et al., 2009). Modern analyzers, such as the differential mobility spectrometer (DMS), have the capability of detecting VOCs in the parts-per-trillion range and the potential to be reduced to portable, field-mobile devices suitable for use in the orchard (Davis et al., 2010; Hao et al., 2009). Should these results be validated, the availability of a practical VOC-based detector of *C*Las-infected trees nevertheless is likely to be several years away.

In hyperspectral imaging, data from a broad section of the visible or visible-plus-infrared spectrum are collected across the image of an object and analyzed to produce a signature of the object that, in the case of a plant, may reflect its physiological state (Aleixos et al., 2002; Du et al., 2004; Blasco et al., 2007; Mishra et al., 2007; Nicolaï et al., 2007; Du et al., 2008; Lee et al., 2008; Qin et al., 2009). Hyperspectral data have been reported to detect changes in the chemical composition of plants (Ferwerda, 2005; Tilling et al., 2007) revealing, for example, the nutrient and water status of irrigated wheat (Tilling et al., 2007). Spectral differences have been detected in comparisons of healthy and HLB-affected citrus using detached leaves and leaves of intact citrus trees (Mishra et al., 2007; Tilling et al., 2007; Lee et al., 2008; Poole et al., 2008). Hyperspectral imaging is very data intensive, and its practical application in an orchard would presumably require the use of multiple detectors, field-rugged computers and sophisticated software packages that may require significant money and time to develop. However, if a few essential components of the hyperspectral image can be identified, far less elaborate spectral analyses may be sufficient to provide biomarkers for HLB. Surface-enhanced Raman spectroscopy (Zeiri, 2007; Kiefer, 2008; Driskell et al., 2009) and laser-induced fluorescence

(Panneton et al., 2010; Malenovsky et al., 2009) are other spectral methods that have found application for detection of plant biomarkers.

In current practice, symptomatic leaf samples are collected from orchard trees suspected of being *C*Las-infected, and the samples are transported to a laboratory for analysis. There is a critical need for compact, user friendly, low-cost, field-deployable instruments or devices for rapid and high-throughput identification of *C*Las-infected trees, symptomatic or asymptomatic, in the orchard. Devices under consideration may rely on simple preparation of extract to be analyzed or on a microelectrode to be inserted into tree tissue. Classes of compounds to be detected include nucleic acids, antigens and small molecules. For example, a research project is aimed at developing a novel oligonucleotide microarray technology that will generate a colorimetric signal after amplification and rapid nucleic acid hybridization. Nucleic acid purification, amplification and hybridization occur in a device about the size of a credit card by lateral flow chromatography on a nitrocellulose membrane (Carter and Cary, 2007). This technology is similar to the home pregnancy test kit, and a prototype was tested in a grove in the fall of 2009 where it detected CTV, the target to which it was designed . Other devices are being developed that are designed to provide cost-effective surveillance for several pathogens or traits simultaneously by relying on multiplexed analyses and computational methods. In higher organisms, small RNAs (miRNA) are often induced rapidly and specifically by invading pathogens, making miRNAs attractive potential biomarkers for early and specific disease diagnosis (Zhang et al., 2006).

The Citrus Transcriptome

The citrus genome sequence, when it becomes available, will provide a list of all of the candidate genes of the citrus tree and the complete amino acid sequences for almost all of the proteins those genes encode. Part of the key to understanding the function of a given gene resides in the molecule that serves to transfer the information encoded in genomic DNA to the cell machinery that synthesizes the protein, i.e., the mRNA molecule. Many of the genes on the candidate list will be so well known from prior genomic and biochemical work with other organisms that functions can be assigned to the corresponding gene products (proteins) with a high degree of certainty. The functions of many other genes will remain a mystery. What will also be almost entirely unknown will be what genes are active in what tissues of the tree and at what stage in development or disease state of the tree. Although protein synthesis is not strictly proportional to mRNA synthesis in any cell, a specific protein will not be synthesized without the corresponding mRNA being present. Thus, identifying what mRNAs are accumulating in what tissues and at what time and under what conditions of stress, i.e., characterizing the transcriptome, can be very informative for various approaches to combating disease or improving productivity. Analysis of the transcriptome and the corresponding global accounting of the proteins of the organism, designated as the proteome, can result in significant new understanding of the etiology of HLB.

Changes in gene expression due to bacterial infection have been observed for citrus, including a few infection-related changes that precede symptom development (Albrecht and Bowman, 2008; Cernadas et al., 2008; Kim et al., 2009), suggesting that it may be possible to develop the desired biomarkers. The technology used in these investigations is the DNA array, or gene chip. Tens of thousands of synthetic short DNA molecules, corresponding to known mRNA sequences of an organism, are immobilized on a surface in a regular array of spots. Fluorescently labeled molecules corresponding to RNA extracted from specific tissue of the organism are exposed to the array. Hybridization reactions result in fluorescent molecules being bound to the array. The pattern of fluorescent spots on the array is interpreted to discover what mRNA molecules accumulate in enhanced or depreciated amounts under specific conditions in specific tissue. The DNA arrays used in these experiments would not have been possible without earlier work by members of the International Citrus Genome Consortium (http://www.citrusgenome.ucr.edu) that identified tens of thousands of "expressed sequence tags" (ESTs, i.e., sequences from mRNAs). More sensitive and comprehensive methods have recently been developed for identifying differences in mRNA accumulation between infected and healthy tissue. In the "RNA-seq" approach, cell RNA is fragmented and the fragments are copied into DNA. High throughput sequencing methods are applied to millions of these DNA molecules to give an in-depth picture of the identities and relative amounts of the RNA molecules of the sample (Wang et al., 2009). One example is gene silencing, wherein a particular gene is "switched off" by machinery in the cell in response to changes in the environment or pathogen invasion.

RESEARCH SUPPORTING CURRENT HUANGLONGBING MITIGATION PRACTICE: REDUCING ASIAN CITRUS PSYLLID ACCESS TO CITRUS

Suppression of ACP by Insecticide Application

Currently much effort is being expended by university, government and private industry researchers with the goal of developing new, more effective and safer insecticides and insecticide application technologies. Equally important is the goal of maintaining the effectiveness of valuable insecticides by delaying or avoiding the appearance of insect resistanct to the insecticide. Costs, effectiveness, run-off in the environment, applicator safety and effects on non-target organisms are other important considerations affected not only by the choice of insecticide but also by application methods. Among the many insecticides registered and recommended for ACP, the only listed soil-applied insecticide that reduces ACP populations on large trees is aldicarb. Reliance on a single insecticide or class of insecticide will increase the likelihood of insect resistance. Furthermore, reliance on aldicarb is also of concern from an environmental and health standpoint. Other alternative insecticides should be used and, in recent years, a suite of insecticides with novel modes of action have been developed. Insecticides are now formally classified into groups based on mode of action (IRAC, 2009). There are newer insecticides (e.g. spinetoram) from new insecticide classes that are recommended for ACP. These products have restrictions on the number of times they may be used so that resistance does not evolve rapidly occur. Other products such as cyazypyr and its derivatives also hold some promise for control of ACP. These groups and still others present many options for controlling ACP, and collecting new information about how they can be most effectively deployed to control ACP is likely to be a major research goal for the foreseeable future.

Success in ACP management will require improvements in monitoring of ACP populations and of the development of ACP resistance to insecticides. Importantly, more extensive incorporation of IPM principles is needed, including development of appropriate insecticide resistance management (IRM) programs. There are programs (such as in cotton in Australia) where good IRM programs have been practiced continuously with the result that some insecticides remain effective for decades. In a “window strategy,” application of only insecticides of a specific class is allowed within a given Citrus Health Management Area during a given time period (e.g., a 2-month window), followed by rotation to an insecticide of another class. Such rotations have been the foundation of the IRM program. Rotation delays the evolution of resistance to any of the insecticides used (Roush, 1989; Forrester et al., 1993). There may be concerns about implementing such a strategy (e.g. several companies promoting their products simultaneously in the same location), but history has shown that a window strategy is effective in countering development of insect resistance to insecticides.

Some insecticides have long residual action, so there is increased concern about the development of insecticide resistance, because insect populations are exposed to sub-lethal doses of the insecticide over a longer period of time. However, Boina et al. (2009) have shown that when citrus is treated with imidacloprid, ACP adults and larvae that feed on the plant may become exposed to sublethal concentrations, and these can have negative developmental and reproductive effects which nevertheless could lead to population reductions over time. Cocco and Hoy (2009) conducted laboratory studies showing that certain adjuvants had the effect of increasing ACP mortality while reducing the mortality of its parasitoid.

Sterile Insect Technique

The sterile insect technique (SIT) applies to pests that reproduce sexually and works effectively if sterilized males are sexually aggressive and successfully compete with indigenous males for mating with females (Knipling, 1955; Knipling, 1985). This method should be considered for use only with a serious pest where thorough knowledge exists of its biology, ecology and behavior and methods have been developed of its mass rearing.

The most commonly used method for inducing sexual sterility in insect pests is by radiation emitted from radioisotopes such as caesium-137 or cobalt-60 (Bushlad and Hopkin, 1953; Lindquist, 1955). The dosage of radiation applied must have no significant adverse effect on the male longevity, searching behavior or mating ability. Matings between sterile males and wild females do not yield offspring. Thus, if sufficient numbers of sterile males are released, most indigenous females will mate with sterile males, the number of individuals in the wild population will be reduced in the next generation. Thus, the concept of SIT uses continued releases of high-quality sterile males to overwhelm wild-type males over successive generations and result in progressively reducing the indigenous population to levels of extinction. If SIT can be developed for a pest, it is the key component of an area-wide integrated pest management program.

SIT has been used successfully with a variety of pests. The most well-known is the screwworm fly, a pest that feeds in open wounds of cattle and other animals. Screwworms were mass-reared and irradiated. Sterile male screwworm adults were then releeased by the millions into populations of indigenous screwworms reduced in size by insecticides (Knipling, 1955; 1985). Pesticide application is necessary to reduce the feral population and increase the proportion of sterile males to indigenous males. The SIT has now also been used against the pink bollworm moth codling moth, false codline moth, painted apple moth, cactus moth and others. Unfortunately, current SIT technology does not appear to be appropriate technique for suppression of ACP. No mass-rearing program has been developed to produce ACP in large enough numbers to make irradiation and SIT feasible. Furthermore, nothing is known about radiation methods to sterilize psyllids or what consequences irradiation and sterility may have on male longevity, flight capacity or mating behavior.

ACP Trap Plant

A trap plant may be of a distinct species from the crop plant, or it could be a peripheral planing of the crop plant. In an example of the latter, a 10-m zone of papaya trees is planted as a trap crop around the main papaya groves, which helped reduce damage from the papaya fruit fly, *Toxotrypana curvicauda* (Aluja et al., 1997). The ornamental citrus relative, *Murraya paniculata* (orange jasmine, mock orange), is known as a preferred host plant for ACP. *M. paniculata* has been considered as a candidate psyllid trap plant but actually is unsuitable because it is also a host for *C*Las. Transformation of this plant with a gene that encodes an ACP feeding toxin has been proposed. However, it is likely that under orchard conditions the proportion of ACP on *M. paniculata* or any other known candidate trap plant would be small relative to the proportion on the commercial citrus trees, which are themselves good hosts of the psyllid. This difficulty might be overcome by pairing trap plants with the use of repellents on citrus (see section below on Distraction of ACP by Chemical Attractants and Repellants).

Guava as ACP Repelling Plant

Several groups of scientists from Australia, the United States, Brazil, France, and Spain that visited the Mekong Delta of South Vietnam have noted that mixed orchards of guava (*Psidium guajava*, Xa Li and Bom cultivars) and citrus (mostly King mandarin, and pummelo cultivars Nam Roi, Da Xanh, and Doan Hung) near My Tho, Vinh Long, and Can Tho have very low populations of ACP and show very few HLB symptoms. In contrast, nearby guava-free citrus orchards have high psyllid populations and high percentages of HLB-affected trees. These observations have been made in what are essentially commercial orchards in collaborations between growers and the South Vietnam Fruit Research Institute (SOFRI) at My Tho. Other plant species (i.e. banana, longan, mango, and durian) have not demonstrated this effect. The guava effect is pronounced only under the following conditions: (i) the main crop and citrus is only used as an intercrop (i.e., typically one citrus tree surrounded by four larger guava trees); (ii) guava trees have been planted and become well-established one year before the citrus trees were planted; and (iii) guavas and citrus are planted close together. The guava affect occurs year-round, which suggests that leaves rather than fruit are the source of protection. The guava-induced protection is not sufficient to keep the psyllids completely out of the orchard, especially once trees become two to three years old, an age when they must be supplemented with one or two insecticide treatments per year. Protection is diminished once the citrus trees are taller than the guavas. The citrus trees themselves may exhibit symptoms of HLB , and the number of HLB-affected citrus trees increases as the mixed orchard reaches three to four years of age. The high ratio of guava trees to citrus trees needed to achieve protection does not seem compatible with current Florida citrus production, but guava trees could find application in orchards following the Advanced Citrus Production System (described below). Field experiments have been set up to test the protective power of guava under Florida conditions.

Distraction of ACP by Chemical Attractants and Repellents

Research at the University of Florida is aimed at identifying and developing attractants for ACP, for both monitoring and management. Male ACP colonize in great numbers on those citrus plants that are currently or were previously infested with virgin or mated female ACP, suggesting that female ACP may produce a sex pheromone that attracts conspecific males, a suggestion supported by olfactometer results (Wenninger et al., 2008). Results of behavioral tests and electrophysiological experiments indicate that the ACP uses both visual and olfactory cues in orientation to host plants (Wenninger et al., 2009b). Analyses by gas chromatography and mass spectrometry indicated that both sexes of ACP produce several volatile compounds, with some compounds specific to each sex. Research is underway to develop methods to use attractants to recruit parasitoids into groves to improve biological control. These results have not yet been published and should be considered preliminary. Infection by specific aphid-transmitted viruses has been shown to produce apparently aphid-attracting VOCs (Medina-Ortega et al., 2009; Werner et al., 2009), suggesting another approach to identifying ACP-attracting volatile compounds.

Protection of citrus from ACP in mixed guava-citrus orchards is described above, research is also underway to develop a repellent for ACP, based on the volatiles released by guava and their effects on ACP behavior. Results from laboratory experiments suggest that the guava effect is due to volatile compounds produced by the guava plants, which may be acting as psyllid repellents, or by masking or counteracting the citrus volatiles that attract the psyllids. Young and old guava leaves are equally active as a source of repellents and male and female psyllids are similarly repelled.

It has been shown that crushed guava leaves were more repellent to ACP than intact guava leaves. Analyses of volatiles from crushed and intact "white" guava leaf flushes were compared (Rouseff et al., 2008). Undamaged guava leaves yielded five sulfur volatiles, and crushed leaves yielded an additional sulfur volatile, dimethyl disulfide (DMDS). Leaves of sweet orange, grapefruit, and rough lemon, crushed or intact, did not give off DMDS, and DMDS is highly repellent to ACP. Sulfur compounds are highly toxic to most insect species due to the disruption of the cytochrome oxidase system of the insects' mitochondria (Dugravot et al., 2004). In olfactometer studies by Noronha and Bento (2008), intact guava plants without crushed leaves repel psyllids, so the protective effect of guava in mixed orchards likely is not due to DMDS. This observation does not diminish the merits of DMDS as a potential psyllid repellent.

Repellent effects of available DMDS formulations have remained for 3 to 4 weeks after distribution of dispensers, which should be long enough for preliminary field studies to assess exclusion of ACP from the groves. If DMDS is effective and new formulations, perhaps with other active ingredients included, can extend the longevity to 15 weeks or more, field trials could lead to registration with the US EPA. Registration will be costly and may require from 8 to more than 55 months, but could result in an elegant short-term form of HLB mitigation.

Trap plants have been proposed for use in conjunction with feeding repellents of ACP compounds such as DMDS in a "push-pull" strategy to concentrate ACP populations into a zone for eradication by insecticide application. Potential feeding repellent compounds include pymetrozine and Flonicamid (Harrewijn and Kayser, 1997; Bedford et al., 1998; Polston and Sherwood, 2003; Bextine et al., 2004; Morita et al., 2007), which have worked for suppression of other pathogen vectors. If feeding on citrus can be sufficiently disrupted, ACP transmission capability may be reduced along with ACP numbers. The push-pull strategy has been considered as a component of an area wide ACP-HLB management program.

HUANGLONGBING EPIDEMIOLOGY AND ASIAN CITRUS PSYLLID BEHAVIORAL ECOLOGY

There remains a great need for more information on HLB epidemiology under Florida conditions; such information could provide the basis for creation of management/mitigation programs or regulatory programs in the state. Some current information on *C*Las spread in the orchard is presented above in the above section on research and mitigation practices. The currently funded projects on HLB epidemiology are listed in Appendix J. Several of these projects are ongoing in Brazil, while others are being conducted in Florida. The specific research topics include transmission (i.e. via seed), effects of control measures on HLB spatio-temporal progress, comparative epidemiology of HLB caused by *C*Las and *C*lam, and alternative hosts/host range of *C*Las.

Understanding and Exploiting ACP Biology

"Psyllids are probably the most benign of the Sternorrhyncha and therefore the least well studied" (Percy, 2010). That is, compared to other hemipterans such as aphid, whitefly, mealy-bug and scale insects (all phytophagous sucking insects), few psyllid species are significant pests of crop and ornamental plants. Moreover, most psyllids have tropical distributions and are found on woody plants (few herbaceous crops and no grains are hosts to psyllids), and therefore tend to receive less attention from entomologists than do non-pest species. What was known about ACP prior to 1998 is from studies of the insect in Asia and islands in the Indian Ocean (Halbert and Manjunath, 2004). Subsequently, entomologists, primarily in Florida, took up the study and the number of publications on ACP expanded exponentially. In his review, Hall, (2008) remarks that "entomologists are in the discovery phase of research detailing information on the biology, behavior, ecology and biological control (a topic of Chapter 2) of *D. citri* in hopes of finding weak points in psyllid populations that could be exploited to help curb disease transmission and reduce the need for chemical control." Research topics have included life history, biology and behavior, host plant relationships, sampling, vector/pathogen relationships, and vector control strategies. Nevertheless, some aspects of ACP have been neglected or have received insufficient attention, as indicated below.

Behavioral Ecology

Study of adult and immature ACP behavior, movement, feeding, *C*Las transmission and other interactions with plant hosts (Wenninger et al., 2009b) could yield information applicable to reducing HLB spread. Spread of HLB in Florida occurs primarily by the dispersal of psyllid vectors, but could potentially be spread by citrus nursery stock including ornamental citrus. Relatively little is known about the flight behavior of ACP. Some reports indicate limited, short-distance dispersal; others suggest longer migratory flights. It is likely that both occur. Extensive information about aphid flight behavior (Vialatte et al., 2007; Lankin-Vega et al., 2008; Klueken et al., 2009) could guide ACP investigations. Information on psyllid flight could facilitate improved sampling methods for monitoring ACP with benefits to management approaches. Although the technology would not be applicable to citrus, an example of an approach that has been successful in reducing transmission of virus diseases has been the development of reflective mulches to repel viruliferous aphids from vegetable crops (Brown et al., 1993; Stapleton and Summers, 2002). Research on the behavior of ACP adults has revealed aspects of probing (stylet penetration) behavior and of communication by means of substrate vibration and semiochemicals (Wenninger et al., 2008; Wenninger et al., 2009a; Wenninger et al., 2009b; Bonani et al., 2010).

The nymphs deserve more attention than they have received because their unique and specialized lifestyle may make them more vulnerable to manipulation than adults. As with other psyllids, ACP nymphs are highly specialized. They are relatively immobile, live closely aggregated and are seemingly vulnerable. Fourth and fifth instar nymphs have been found to acquire *C*Las efficiently and become, as adults, inoculators of HLB. ACP nymphs almost always are found on the new flush of growth of their hosts. Psyllids likely have evolved behavioral tactics to protect themselves in the developing colony.

Two strategies employed by other hemipterans whose nymphs are closely aggregated are alarm pheromones (Nault and Phelan, 1984) and myrmecophily (beneficial association with ants) (Hölldobler and Wilson, 1990). Aphid alarm pheromones have been used to manage aphid pest populations (Dawson et al., 1990; Guedot et al., 2008), but no studies report a search for alarm pheromones in any psyllid species. There are a few anecdotal reports of ants collecting honeydew from ACP nymphs and from the nymphs of other psyllid species, suggesting the possibility of facultative myrmecophily. Chemical communication between ants and their attendant hemipterans (Nault et al., 1976) could lead to the discovery of new semiochemicals and novel approaches to reducing ACP populations. There may be predator and parasitoid avoidance vibrational signals (Cocroft, 2001; Wenninger et al., 2009a) and evasive behaviors that are critical to nymph survival. Modifications of host leaves induced by salivary secretions (Luft et al., 2001), recruitment of ants by nymphs to avoid predators (Novak, 1994) and chemical (or tactile) communication between ants and psyllids are other aspects of nymph behavior that could inspire new control measures in the longer term.

Two aspects of ACP nymphal development deserve close attention. The first is the ability of the immature psyllids to alter the biochemistry and morphology of their hosts and create the protective niches in which they live. Developing plant tissues may be responding to compounds in ACP saliva. Isolation and characterization of these putative chemicals could lead to new ways to alter psyllid populations as has been suggested by Miles (1999) for aphid salivary enzymes. The second subject concerns ACP nymphs' incorporation of their honeydew into non-sticky, waxy filaments that are readily carried away from colonies by wind and rain. Learning more about the chemistry of ACP excreta would be worthwhile. In addition to protecting nymphs from fouling their environment, the excreta may contain compounds that protect them against predators, parasites and pathogens, or conversely volatiles that attract the enemies of predators and parasites. Such compounds could lead to new ACP management strategies.

Vector-pathogen Interactions

Information on *C*Las transmission rates and *C*Las effects on ACP is needed to provide the foundation for epidemiological models and predictions of HLB spread and other HLB investigations. However, published reports on *C*Las transmission by ACP are not in agreement with transmission rates from either field-collected insects or laboratory-reared insects. This uncertainty may be attributed in part to variations in experimental conditions, e.g., the use of different plant species and cultivars (Xu et al., 1988). However, at this point the data are too few to allow underlying variations in transmission to be distinguished from variation due to experimental method, and more data are needed.

Presently, little attention is being given to the potential impact of *C*Las infection on the fitness of the ACP vector. In other systems, plant pathogens have been found to decrease or to increase the lifespan and fecundity of vectors carrying circulative, propagative pathogens. Infection of the plant host may make the plant more beneficial to the feeding insect vector, expand the host range of the vector, or improve its survival under an environmental stress such as over-wintering (Purcell and Nault, 1991). In natural ecosystems, well-adapted plant hosts and vectors of pathogens are not harmed by infections (Caudwell, 1984). Almost certainly HLB propagates in ACP, and ACP may be the ancestral host for *C*Las. A long *C*Las-ACP association would predict that *C*Las is not pathogenic to ACP and could be beneficial (Purcell and Nault, 1991), whereas an association at a more primitive evolutionary stage may result in pathogenicity (Purcell and Suslow, 1987).

Transovarial transmission (vertical transmission from parent to offspring) and sexual transmission are vector-pathogen phenomena that could influence HLB epidemiology. Experimentally demonstrating transovarial or sexual transmission requires care to insure that plant hosts for rearing ACP are *C*Las-free or non-susceptible (Purcell, 1982). van den Berg et al. (1992) reported some evidence that a HLB agent is transmitted transovarially and/or during oviposition or that greening can be acquired by plants via the eggs of the psyllid *Trioza erytreae*. More recently, Hansen et al. (2008) reported that bacterium associated with *Ca*. Liberibacter pysllaurous/solanacearum (*C*Lp/s) is transovarially transmitted by the potato psyllid, *Bactericera cockerelli*. The 16S rRNA sequence of *C*Lp/s has 97 percent homology to *C*Las from Brazil, and *B. cockerelli* is in the same taxon as *T. erytreae*, so transovarial transmission of *C*Las could occur with ACP. If vertical transmission of *C*Las occurs at all, the extent apparently is very limited and unlikely to be of significance in a full blown epidemic with high ACP populations. However, where both sources of *C*Las inoculum and ACP have been reduced to low levels, vertical or sexual transmission could make destroying the last vestiges of *C*Las more difficult.

RESEARCH ON CITRUS BREEDING AND GENETICS

Citrus Breeding

Plant genetic improvement through both conventional and genetic engineering (GE) approaches has been a primary strategy to develop improved agricultural crops. In conventional breeding programs, scientists cross or interbreed close and even distantly related species to develop new crops with agronomically desirable phenotypes. However, as demonstrated by over 50 years of international research efforts, breeding of improved germplasm to address HLB has been technically daunting due to the challenges inherent to the HLB bacterial agent, the vector, and, most importantly, the inherent difficulties in breeding with citrus germplasm. Citrus improvement through conventional approaches is limited mainly because of the fruit's complex reproductive biology. Therefore, a statistically predictable breeding program, based on normal sexual reproduction, is not available for many commercial citrus species.

Citrus efficiently forms apomictic (nucellar) embryos. That is, apomixis (asexual seed formation, termed as nucellar embryony in citrus) bypasses sexual reproduction and interferes with developing a traditional breeding program for many citrus species. Nucellar embryos arise from the maternal nucellar tissue and thus are clones of the maternal parent. Nucellar embryos initiate prior to pollination and are more vigorous than zygotic embryos resulting in very few zygotic embryos from a cross. With the exception of pummelo, citron and some mandarins, most citrus species such as lemon, lime, sweet orange, grapefruit, and mandarin exhibit varying ranges of apomixis. Juvenility is another factor affecting breeding programs. Citrus typically requires 3–7 years to reach flowering and maturity. The long breeding cycle, coupled with the large size of the trees, greatly limits the number of families and individuals that can be evaluated in a breeding program. Finally, heterozygosity, or the presence of different alleles at a locus, is very high in most citrus clones and results in substantial and unpredictable genetic segregation in progeny (Moore, 2001).

As a consequence of its unique reproductive biology, most of the scion and rootstock cultivars in commercial production were not developed through systematic breeding programs, but rather as spontaneous seedling or bud sport mutations (Talon and Gmitter, Jr., 2008). There has been some success, however, in breeding for citrus rootstocks. For example, intergeneric crosses between *Citrus* and *Poncirus* sp. and the several resulting cultivars “Carrizo”, “Troyer” and “Swingle”, while not acceptable as scions, are widely used as rootstocks because of their resistance to soil diseases and nematodes (Soost and Roose, 1996; Moore, 2001).

Conventional plant breeding for resistance to HLB is likely to be extremely challenging. A dominant gene for resistance to *Citrus* CTV is known in *Poncirus trifoliata*, and species highly resistant to citrus canker, in calamondin, kumquat and *Ichang papeda* also exist. However, there is little or no evidence for genetic resistance to HLB in *Citrus* or related genera and a survey in South African orchards, where HLB has been present for decades, failed to identify any HLB-disease resistant trees (Roux and Grout, Citrus Research International, Nelspruit, South Africa, personal communication). Even with good sources of HLB resistance, the most optimistic timelines suggest that a minimum of eight years would be required to develop resistant mandarins and a substantially longer time for the difficult breeding systems of oranges or grapefruit (Roose and Close, 2008).

Somatic Hybridization of Citrus

In the 1980s, the complications limiting conventional breeding for new citrus cultivars were thought to be overcome by the development of somatic hybridization by protoplast fusion. These techniques circumvented sexual incompatibility and allowed crossing to create interspecific hybrids through *in vitro* fertilization. Citrus somatic hybridization has been successfully used in citrus to produce over 200 parental combinations and to generate hybrid rootstock which are under field evaluation (Grosser et al., 2000). However, the variety of progeny produced by somatic hybridization is more limited than those produced in sexual crosses. Somatic hybridization has yet to fulfill the commercial hopes of generating new cultivars to meet the increasing agronomic needs of the citrus commodity.

Citrus Genetic Transformation

Genetic transformation of citrus for resistance to *C*Las or ACP provides a viable alternative to the technically challenging conventional breeding of the most widely planted citrus species. GE has the potential to introduce disease resistance trait(s) with negligible alteration of the integrity and traits of commercially important scion clones. Genetic transformation of citrus was first reported in the early 1980s. Since then, citrus has been transformed employing *Agrobacterium*, chemically induced DNA uptake into protoplasts, and particle bombardment (Peña and Navarro, 1999; Singh and Rajam, 2009). The first variety of transgenic sweet orange (Washington navel) was developed in Japan by *Agrobacterium*-mediated transformation and regeneration of citrus from suspension cells (Hidaka et al., 1990). Gloria Moore in Florida has been another pioneer in the development of *Agrobacterium*-mediated transformation of citrus (Moore et al., 1992). Thus far a number of major citrus crops have been transformed with genes of potential agronomic importance (Table 3-1).

TABLE 3-1 Major Citrus Species that have been Genetically Transformed to Produce Citrus Plants with Genes of Agronomic Interest

Common Name	Scientific Name	Gene Introduced	Reference
Carrizo citrange	*Citrus sinensis* × *Poncirus trifoliata*	Citrus blight-associated gene	Kayim et al., 2004
Carrizo citrange	*C. sinensis* × *P. trifoliata*	HAL2 gene	Cervera et al., 2000
Carrizo citrange	*C. sinensis* × *P. trifoliata*	LEAFY and APETALA1	Peña et al., 2001
Grapefruit	*C. paradisi*	Carotenoid biosynthetic genes	Costa et al., 2002
Grapefruit	*C. paradisi*	Coat protein gene of *Citrus tristeza virus* (CTV)	Moore et al., 2000
Grapefruit	*C. paradisi*	CTV genes	Febres et al., 2003
Mexican Lime	*C. aurantifolia*	Coat protein gene of CTV	Domínguez et al., 2000
Ponkan mandarin	*C. reticulata* Blanco	Chimeric ribonuclease gene	Li et al., 2002
Rangpur Lime	*C. limonia*	bO (bacterio-opsin)	Azevedo et al., 2006

Common Name	**Scientific Name**	**Gene Introduced**	**Reference**
Sour orange	*C. aurantium*	Coat protein gene of CTV	Gutiérrez-E et al., 1997; Ghorbel et al., 2000
Trifoliate orange	*P. trifoliata*	Capsid polyprotein gene (pCP)	Iwanami et al., 2004
Trifoliate orange	*P. trifoliata*	Citrus FT (CiFT)	Endo et al., 2005
Trifoliate orange	*P. trifoliata*	Gene encoding human epidermal growth factor (hEGF)	Kobayashi et al., 1996
Trifoliate orange	*P. trifoliata*	rolC gene	Kaneyoshi and Kobayashi, 1999
Troyer citrange	*C. sinensis* × *P. trifoliata*	Truncated version of CTV and Bar gene	Piestun et al., 2000
Troyer citrange	*C. sinensis* × *P. trifoliata*	rolABC genes	Gentile et al., 2002 Cirvilleri et al., 2005
Valencia orange	*C. sinensis*	Pectin methylesterasegene	Guo et al., 2005
West Indian lime	*C. aurantifolia*	Genes for decreased seed set	Koltunow et al., 2000

Source: Extracted from Singh and Rajam, 2009.

In order for GE to become a practical strategy in citrus, the transformation efficiency needs to be increased, the extended regeneration times need to be reduced, and the extended juvenility period needs to be shortened. Most published *Agrobacterium*-mediated transformation procedures begin with juvenile citrus material such as epicotyl segments from seedlings, which is favored as being more amenable to transformation (Bond and Roose, 1998; Ali and Mirza, 2006). The result is juvenile transgenic citrus plants with delayed time, usually years, to flowering and acquisition of mature growth habits. Plants derived from such protocols must be evaluated not only for their resistance traits but for being true-to-type and having desired horticultural characters. In contrast, using well-characterized citrus varieties as the mature citrus starting material minimizes the time to flowering and the need for horticultural evaluation.

Methods for transformation of mature citrus material, including sweet orange, were developed in Spain by Peña and colleagues (Cervera et al., 1998; Cervera, 2005). Using this approach, which relies on supervirulent *Agrobacterium* strains and careful selection of starting material, the juvenile stage was bypassed, effectively reducing the time to fruit-set to 14 months. Peña and colleagues have transferred this material and the procedure to São Paulo State, Brazil, where they have produced mature transgenic citrus trees expressing antibacterial peptides, which now are under evaluation. A critical factor in mature citrus transformation is collection of starting explants from vigorous, optimized citrus trees having a very low population of contaminating bacteria and fungi.

A coordinated and focused effort to develop mature citrus transformation systems is underway in Florida in several institutions or laboratories. Emphasis is put on citrus plant pre-conditioning and parameters affecting transformation and regeneration. Also, to gain time, the transformation technology for mature citrus tissues, which has been developed in Spain, will be transferred to Florida, where new laboratory and greenhouse facilities are being established.

Because mature tissue transformation is not yet available in Florida and juvenile citrus transformation is more efficient and less cultivar specific than mature citrus transformation, a project on accelerating the production and commercialization of transformed juvenile citrus is also being developed. Several approaches have been taken to minimize or eliminate the problem of juvenility. These include use of high-quality sweet orange clones selected for reduced juvenility and development of a universal transgenic rootstock that promotes flowering by expression and transport into the scion of a flowering-inducing protein. Another strategy for curtailing the maturation period of juvenile citrus is transformation with genes that accelerate flowering, such as the Arabidopsis *LEAFY* or *APETALA* (Peña et al., 2001).

Attention is being given to another approach to introducing resistance to *C*Las into varietal scions: genetic transformation of citrus rootstock. If expression of the transgene results in the production of a resistance-mediating molecule that can be secreted in the rootstock and effectively mobilized across the graft union, the rootstock could be used to confer resistance or tolerance to a variety of non-transgenic, fruit-producing citrus scions. Use of mature scions would avoid the long juvenility phase.

TRANSGENES FOR RESISTANCE TO *C*LAS

In order for GE of citrus to contribute to HLB mitigation, transgenes capable of conferring resistance to *C*Las or ACP or tolerance to *C*Las must be identified and incorporated into constructions suited for expression and proper targeting in citrus. Several research groups are working on the isolation and testing of candidate genes.

Anti-bacterial Peptides

Anti-bacterial peptide (ABP) genes have received early attention because this class of molecules represents one of the few known with members capable of acting against *C*Las and other bacteria. ABP genes can be found in humans and animals (<u>Myeloid antimicrobial peptides, human beta-defensin-1</u>, <u>Polyphemusin-1</u>, <u>Tachyplesin</u>, <u>Protegrin-1</u>, <u>magainin</u>, <u>indolicidin</u>, and others), insects (<u>cecropins</u>, <u>Sarcotoxin IA</u>, <u>pyrrhocoricin</u>, and other), and plants (<u>defensins</u>, and others). The underlined peptides are examples of those that have already been evaluated in citrus against the liberibacters.

In 2009, field trials for transgenic citrus were established in Florida. Currently under evaluation for resistance to citrus canker and HLB are (i) a Carrizo citrange that carries a gene that encodes for a plant-based anti-microbial peptide, being tested by Southern Gardens Nursery LLC and Texas A&M University; and (ii) Carrizo citrange and grapefruit that carry a proprietary gene called 'Disease Block"(i.e. a phage-based protein that disrupts the outer membrane of Gram negative bacteria but does not lyse the cells), being tested by Southern Gardens and Integrated

Plant Genetics. No field resistance data have been generated as of December 2009 but preliminary laboratory data for the transgenic citrus indicate efficacy against citrus greening (M. Irey, United States Sugar Corporation, Clewiston, FL, personal communication).

Genes Involved in Systemic Acquired Resistance (SAR)

SAR is an important defense mechanism in plants which, when activated by a specific microorganism or chemical, can provide protection against a variety of challenging pathogens. The Arabidopsis NPR1 gene is induced in SAR and in turn NPR1 induces the expression of a battery of downstream pathogenesis-related (PR) genes. Over-expression of NPR1 in Arabidopsi*s*, as well as in rice and tomato, induces broad spectrum disease resistance. Constitutive expression or over-expression of NPR1 and other SAR genes in transgenic sweet orange is now being evaluated for their ability to confer resistance to *C*Las and other bacteria, with the possibility of producing broad-spectrum resistance. One concern about SAR proteins is they may adversely affect yield. Since the SAR transgenes are of plant origin, should they be found to be effective, there should be less regulatory concern than would be found for transgenes from other organisms.

RNA Interference to Limit Psyllids

Recently, plant-mediated RNA interference (RNAi) has been shown to effectively control plant insect pests, at least in the laboratory settings. Transgenic corn plants expressing corn rootworm double-stranded RNAs (dsRNAs) exhibited a significant reduction in rootworm damage, and they induced mortality of the insects (Baum et al., 2007). These dsRNAs, after being ingested by the insect, were processed in the insect into small RNAs. The natural gene silencing mechanism of the insect directs destruction of sequences corresponding to those in the dsRNAs in the target gene, leading to death of the insect. Similarly, transgenic cotton plants expressing the dsRNA of a cotton bollworm p450 mono-oxygenase gene effectively inhibited cotton bollworm. Therefore, there is a potential for using RNAi as a control measure for psyllids, and projects taking this approach are in progress. Essential genes for psyllid development or viability could be chosen for RNAi in citrus trees.

As the various anti-*C*Las or anti-ACP genes are created, there will be a need to test them in transgenic citrus. Many of the programs that are investigating such genes do not have capabilities for producing transgenic plants. There will be an increased need for the production of transgenic material and the facilities presently available may not be able to meet those needs. The Core Citrus Transformation Facility (CCTF) at University of Florida Citrus Research and Education Center (UF-CREC) is a service laboratory open to qualified investigators. With additional funding for employees and equipment, the CCTF could increase its production significantly to meet the needs for transgenic citrus.

TRANSGENIC, VIRAL, AND BACTERIAL DNA VECTORS FOR MEDIATING GENE EXPRESSION IN CITRUS

There is a general consensus that the most viable long-term approach to HLB mitigation is to deploy trees that are resistant to *C*Las, or ACP, or both . Intensive research efforts in Florida are devoted to this long-term objective, which will likely require years of effort. Resistant plants can be produced by (i) classical genetics and plant breeding, (ii) genetic transformation to produce transgenic plants, and (iii) introduction of a bacteria- or virus-derived DNA vector bearing a gene of interest. In an example of the latter case, *Agrobacterium tumefaciens* Ti plasmids are commonly used in plant research to express genes in pressure-infiltrated leaves, for example, without the need for time-consuming genetic transformation. Plant viruses are another source of DNA vectors for tests of gene expression without transformation.

Usually, use of bacterium- or virus-mediated gene expression is termed “transient expression” because the longer term, multi-generational gene expression characteristic of genetic engineering is not observed. It is the good fortune of citrus research, and possibly the citrus industry, that W.O. Dawson of UF-CREC has developed CTV, itself a significant disease agent under specific circumstances in Florida, into a viral gene expression vector. This CTV vector was found, very surprisingly, to maintain gene expression for years.

Citrus Tristeza Virus-Mediated Systemic Expression of Foreign Genes in Citrus

The CTV vector is likely to find application because of the urgent need to have HLB mitigation measures in place in less time than the 10–15 years, after a functional anti-*C*Las gene has been identified. The latter would be required even for the most facile transformation of mature citrus tissue. The CTV-based vector is now being developed as an interim measure to protect citrus trees against HLB without the (as yet unavailable) long-term transformation procedures. That is, it appears that CTV vectors may be used not only to test transgene constructions for later genetic transformation into citrus but also possibly for “transient” expression of transgene constructions over a period of several seasons in the orchard.

The CTV vector is applied without transformation, without regeneration, and without the use of a selectable marker or reporter gene. The CTV-based vector allows insertion of one, two or three genes into the viral genome, and these inserted genes are expressed together with virus genes as the virus vector replicates in the host plant. The vector can express high levels of the products from the inserted genes, systemically in both shoots and roots. Most interestingly, after having been amplified within an initial citrus tree, the CTV vector can be transmitted by graft-inoculation to other citrus trees of any size or variety.

The longevity of CTV-mediated gene expression is truly surprising. So far, the vector has continually expressed foreign genes in citrus for six years. Twenty-seven of 30 citrus trees, which have been infected with the vector for 4–6 years, still contain the intact foreign gene. Based on this observation, it is very likely that a high percentage of the trees will retain the foreign peptide gene for ten years or more.

CTV is endemic in many countries of Asia, Africa, America, Australia, as well as in some Mediterranean countries, as well as Florida, and citrus trees grown in these regions are tolerant to the virus. Problems arise from CTV primarily where citrus is grown on sour orange rootstock, which rootstock is now avoided in Florida citrus orchards. Thus, using a CTV-based gene vector to infect citrus trees should not harm the trees. In summary, the advantages of the CTV-vector approach, in comparison to the transformation procedures for the production of transgenic trees from juvenile or mature tissues, are:

- A CTV-vector would allow *C*Las-resistant trees to be available in much less time than would be required for production of transgenic trees from juvenile or mature citrus tissues.
- If one or a few trees that are supporting the vector containing the beneficial gene for HLB control become available, the vector construction can be transferred to other citrus trees using vector-infected budwood or rootstock in the nursery or orchard.
- The problems associated with transformation of juvenile tissue are avoided because juvenile trees infected with the vector can be a source of budwood to transfer the vector to mature trees.
- Similarly, budwood from a CTV-vector-infected tree can be transferred to almost any citrus variety, whereas with genetic transformation the time-consuming and expensive transformation protocol would need to be repeated with each new citrus variety.
- The CTV vector bearing an anti-*C*Las construction might be sufficiently potent to be applied not only to healthy trees as a prophylactic but also therapeutically to already infected trees by graft inoculation.
- The CTV vector allows expression of more than one gene, so a properly designed construction could be effective against more than one disease agent.
- Vector constructions that are compatible have been developed, which should allow re-inoculation of the same tree should a first construction fail or become obsolete.
- The CTV-vector infected trees are not transgenic, so there is virtually no possibility of transgene transfer through pollen. However, CTV is aphid-transmitted, so aphids could spread the genes inserted into CTV if virus particles are formed. It should be possible to construct CTV vectors that will not result in gene transfer by aphids.
- CTV is phloem-limited as is *C*las and thus, anti-bacterial peptides or other genes would be expressed in the tissue where the pathogen is located.

Screening of Anti-Bacterial Peptides using CTV-based Vectors

The CTV-vector system is suited to the testing of candidate genes intended to act against any citrus-infecting disease agent (Dawson et al., 2005), but it is particularly well-suited to screen for the effectiveness of anti-*C*Las gene products. No long-term culture of *C*Las has been demonstrated, so conventional *in vitro* antimicrobial screening methods are not applicable. The CTV vector and *C*Las occupy the same site in the host plant, the phloem sieve tubes, and thus the

CTV-vector-encoded anti-bacterial product is produced in, and targeted at, the site inhabited by *C*Las where the liberibacters live and multiply. In practice, a citrus budwood line expressing an anti-bacterial peptide from the infecting CTV vector is propagated in various citrus cultivars including sweet orange. Infection by the CTV vector is confirmed by an enzyme linked immunosorbent assay or ELISA. The plants are then graft-inoculated with a citrus source of *C*Las and subsequently observed for symptom development and tested for *C*Las accumulation. In this way, over 100 genes for ABPs are being screened. Promising candidate peptides already have been identified by the CTV work.

CITRUS CULTURAL PRACTICES MODIFIED TO ACCOMMODATE ENDEMIC HUANGLONGBING

Advanced Citrus Production System

The Advanced Citrus Production System (ACPS) is now being evaluated in Florida citrus groves for potential in maintaining sustainable and profitable citrus production in the presence of HLB and citrus canker. The aim of the ACPS is to shorten and enhance the citrus production cycle. Economic fruit production is expected to occur in as little as 3 years from planting, thus achieving an early return on investment. The intended early high yields are anticipated based primarily on high density planting (conventional grove: 116 trees/acre; high density grove: 363 trees/acre) and optimum nutrition-water relations using intensively managed computerized daily fertigation (fertilizer included in irrigation water), commonly referred to as “Open Hydroponics System” (OHS). The OHS will have to be adapted for Florida summer rainy season and sandy soil conditions. Several field experiments using newly planted and mature trees are in progress to evaluate the ACPS for field plot layouts, spacing, automated fertigation system design, equipment selection, irrigation scheduling, monitoring, remote control, fertilizer formulations, root density distribution, and girdling to enhance cropping and control tree vigor. Because ACPS involves higher tree densities, automated irrigation, and intensive nutrient management, annual cultural costs will increase. Estimates are available of the changes in these costs, as well as a determination of the required yield increase that a grove with an ACPS must achieve to be profitable.

Induction of Systemic Acquired Resistance and Supply of Micronutrients

In Florida, imidacloprid and aldicarb are the only two available systemic insecticides which provide effective control of psyllids on young non-bearing trees. Based on reports that imidacloprid can induce systemic acquired resistance (SAR, described above) to citrus canker and because of its intensive use for psyllid control in newly planted citrus trees, there is interest to determine whether imidacloprid is able to induce SAR against HLB. Experiments are currently underway to evaluate the effect of imidacloprid, as well as non-insecticide SAR inducers, on HLB symptom expression and *C*Las titers in citrus tissues.

In other research, chemical inducers of SAR, such as isonicotinic acid (INA) and acibenzolar-s-methyl (ASM, Actigard®), are being investigated for efficacy against HLB. Initial experiments in which a SAR inducer was applied as a drench did not result in protection against budwood-inoculated *C*Las. Further experiments are aimed at testing SAR inducers under the presumably less intense inoculation method that a citrus orchard would experience when exposed to psyllids.

Curative levels of micronutrients have also been credited with eliminating mineral deficiency symptoms associated with HLB. Commercial-scale, replicated experiments are underway to test the effect of a combination of micronutrients and SAR inducers, with and without insecticidal psyllid control, on the spread and impact of HLB. Growers hope that the putative effect of SAR inducers and micronutrients will make removal of HLB-infected trees unnecessary. However, even if there is such an effect, the trees will not be cured and may continue to serve as sources of inoculum.

MODEL SYSTEMS AND CHEMICAL SCREENING

Two projects underway are using three model system approaches to understanding and combating HLB. In the first approach, compounds capable of suppressing *C*Las accumulation and their effects are being discovered by mass screening of plantlet cultures initiated from *C*Las-infected citrus and periwinkle. The source of compounds is a chemical library similar to the type used in the pharmaceutical industry for drug screening. Each plantlet is exposed to a different compound and tested for reduction in *C*Las titer and possible phytotoxic affects. Two approaches in another project rely on results from the model plant Arabidopsis. The Arabidopsis regulatory protein MAP kinase kinase 7 (MKK7) enhances basal resistance to pathogens and induces SAR in Arabidopsis. Both the Arabidopsis *MKK7* gene and the corresponding gene from citrus are being over-expressed in citrus, which will be tested for resistance to *C*Las (and the canker organism). In the second approach, citrus treated with a mutagen is to be screened for mutants that show elevated activity of the oxidative pentose phosphate pathway (oxPPP), a condition that, in Arabidopsis, results in enhanced disease resistance.

SUMMARIES OF EXPERIENCES WITH OTHER MAJOR PLANT DISEASES

There are many plant diseases which, like HLB, present a serious threat to, and could even eliminate production of, a specific crop. Appendixes L and M summarize the disease and the actions taken in attempting or accomplishing mitigation for two such examples.

AN OVERVIEW OF RESOURCES FOR CITRUS PEST MANAGEMENT PROJECTS

Research that is focused on Florida's pest problems is performed by state-funded personnel using facilities at Florida's Land Grant University (University of Florida) and by staff of the US Department of Agriculture's (USDA) Agricultural Research Service (ARS), using in-house funding at its facilities located in Florida. ARS' in-house funds can be used to foster research partnerhips via specific cooperative agreements with outside entities such as universities and industry (http://www.ars.usda.gov/is/np/SpecCoopAgreements/ SpecCoopAgreementsintro.htm).

The Animal and Plant Health Inspection Service (APHIS), another unit of USDA, also provides funding citrus research infrastructure and personnel. These include the Center for Plant Health Science and Technology (CPHST) and the Citrus Health Response Program (CHRP). These resources provide the long-term infrastructure necessary to deal with pest management problems. There are also citrus/citrus pest research programs in Parlier, California; Frederick and Beltsville, Maryland; and Weslaco and College Station, Texas and these can have an impact on citrus in Florida (www.aphis.usda.gov).

Citrus pest management research is supported by several important national, regional, state and industrial entities. As with many agricultural systems, citrus researchers have found support from USDA in its various programs which include:Agriculture and Food Research Initiative (AFRI) competitive grants program, which replaced the long standing National Research Initiative (NRI) program and the much shorter-lived Initiative for Future Agriculture and Food Systems (IFAFS). These programs have provided major funding for citrus research. AFRI funds programs that advance "fundamental sciences in support of agriculture." (http://www.csrees.usda.gov/fo/agriculturalandfoodresearchinitiativeafri.cfm).

The Pest Management Alternatives Program provides support for and encourages "the development and implementation of integrated pest management (IPM) practices, tactics, and systems for specific pest problems…." (http://www.csrees.usda.gov/fo/pestmanagementalternativessrgp.cfm).

Integrated Pest Management funds projects that "develop and implement new ways to address complex pest management issues". (http://www.csrees.usda.gov/integratedpestmanagement.cfm).

The Interregional Project Number 4 (IR-4) "provides expert assistance with product development and registration for minor crops" such as citrus (http://www.csrees.usda.gov/fo/ir4minorcroppestmanagement.cfm).

In addition to these longer-standing USDA programs, new programs have been more recently established, including the large Specialty Crops Research Initiative (SCRI). SCRI was established to "solve critical industry issues through research and extension activities and gives priority to projects that are multistate, multi-institutional, or trans-disciplinary; and include explicit mechanisms to communicate results to producers and the public". (http://www.csrees.usda.gov/fo/specialtycropresearchinitiative.cfm).

In addition to these USDA programs that cover the broad landscape of agriculture, there were, in the past, special research grants programs specific to citrus, such as the *Citrus tristeza virus* (CTV) research program (http://www.csrees.usda.gov/fo/fundview.cfm?fonum=1079).

An important third level of funding for pest management research in Florida is that supplied by the Florida citrus industry. For example, Southern Gardens Citrus has planted the first research field trials of potential canker and greening disease-resistant citrus trees in its Hendry County citrus groves. The trees, noted to be resistant to canker and greening in the laboratory, were planted in small plots to determine if the trees are disease resistant under commercial grove conditions. (http://www.growingproduce.com/news/flg/?storyid=2047).

Florida, California and Arizona also have also funded research. Prior to the detection of HLB in Florida, funding from citrus producing states was directed towards research that pertained to their own industry's needs. With HLB now present in Florida and with the possibility of HLB reaching other citrus-producing states, industry representatives from Florida and California have begun to discuss combining their financial and laboratory resources in order to arrive at HLB management strategies as soon as possible. In 2008, representatives from Florida traveled to California to learn how the California research funding programs are structured. Most recently, industry representatives met in Dallas, Texas in December 2009 to identify citrus research needs on a national level and to discuss how federal funding could be best allocated. In addition, growers in both Florida and California will vote on the potential establishment of a national citrus research program. All citrus growers will be assessed on a per box of harvested fruit basis to fund this program. Management of this program will be by a board composed of representatives from all citrus-producing states. This vote is planned to take place in 2010.

Over $4 million of the California Citrus Research Board (CRB) annual budget is directed towards HLB and ACP. Approximately $2 million is allocated to research and an additional $2 million funds an operational program that monitors and traps ACP in production citrus groves and supports a high-throughput diagnostic laboratory.

It is important to note that these USDA funding agencies do not have a long-term strategic plan for pest management specific to citrus in Florida. Furthermore, it appears that there are no coordinated long-term plans across agencies for citrus research and extension.

RECENT HISTORY OF INDUSTRY FUNDING IN FLORIDA

The Florida Citrus Production Research and Advisory Council (FCPRAC) has operated since 1991 under the Florida Citrus Production Research Marketing Order (FCPRMO). The order allows growers to tax themselves up to 1 cent per box of citrus and direct those funds to research through a competitive grants program with the Board of FCPRAC making the funding decisions. Historically, this tax raised about $1.5 million annually and, in 2007/08, nearly all of these funds were spent on HLB and canker. In 2007/08, the state provided a grant of $3.5 million and this was combined with $1.5 million from FCPRAC and $2 million from the Florida Department of Citrus (FDOC), the latter being a state agency that is funded by an excise tax placed on each box of citrus that moves through commercial channels. The $7 million of funding in 2007/08 funded 100 projects that were reviewed by FCPRAC, with about 50 percent of the projects going to IFAS and 40 percent to ARS.

In the fall of 2007, industry started an effort to convince the Florida Citrus Commission (FCC), a 12-member board appointed by the governor to oversee the FDOC, that a significant research investment (e.g. $20 million) from the Citrus Advertising Trust Fund (CATF) and other sources was needed to deal with HLB. On January 16, 2008, the FCC passed a resolution that '… expresses willingness to provide support … with expectation of accountability and participation' and on January 28, the Florida Citrus Industry Research Coordinating Council's (FCIRCC) Greening Research Task Force formed the Greening Research Oversight Committee to start development of a comprehensive Research Management Plan.

In early January 2008, representatives of the Florida citrus industry formed an ad-hoc committee to determine a strategic research plan for dealing with HLB in Florida. They examined the Pierce's Disease Program in California to determine how it operated for the coordination and management of a large strategic research and extension program. The group then hired Tom Turpen from the Technology Innovation Group, Inc. (TIG), a consulting group that "promotes the diffusion of innovation through commercialization". In February 2009, they met with the National Research Council (NRC) to suggest forming an expert panel that will develop a list of HLB research priorities and with ARS to form a SWAT-Team for the future of the Florida Citrus Industry. These meetings and interactions resulted in creation of a Greening and Canker Research Management Organization. The conceptual model and the various members and their relationships are diagrammed in Figure 3-1

The Greening and Canker Program was focused on:

1) Urgently finding solutions to production problems, with an emphasis on Greening and Canker but with the realization that other problems will arise in the future.

2) Providing accountability and responsibility for projects and maintaining grower control of the program.

3) Communicating and coordinating activities to stakeholders.

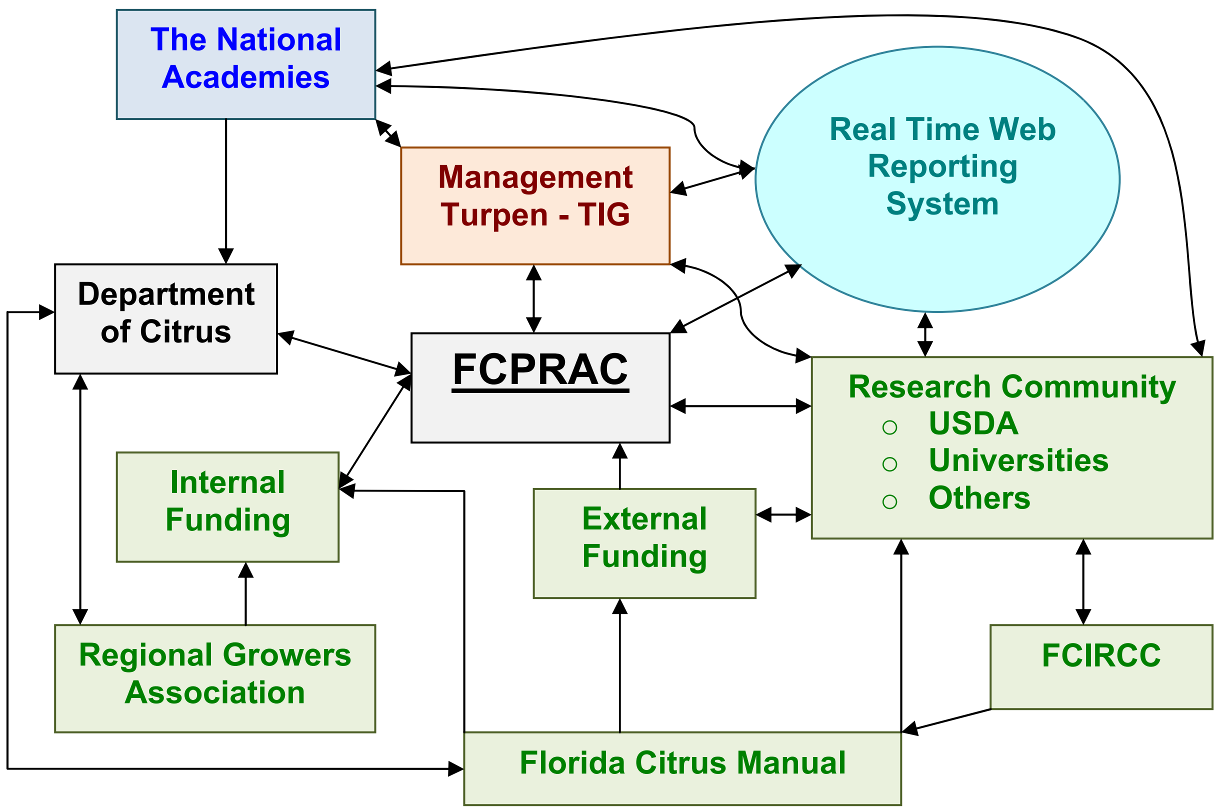

FIGURE 3-1 Conceptual model of agency relationships.
Diagram courtesy of R. Norberg, Florida Department of Citrus, Lakeland, Florida.

FLORIDA CITRUS ADVANCED TECHNOLOGY PROGRAM

A major jump in funding citrus research for Florida was achieved when FCPRAC launched the Florida Citrus Technology Program (FCATP) in 2008, which was the core of what was envisioned in the diagram above. The stated objective of the program is “to apply new technologies to improve the profitability of the Florida citrus industry”. The NRC was commissioned to handle the review of the proposals that were submitted to the FCATP Grants Program in 2008. A total of 205 proposals were received by the FCATP and an initial 83 projects were selected for funding. The total amount of funding awarded was $16,298,243. The target for research funding in 2009 is $34,700,000.

A key element to the FCATP Grants Program should be accountability. In the 2008 RFP, the reporting requirements include a 1-page quarterly report with a more complete annual report stating “progress towards stated milestones…and next steps toward field application”. These are considered to be minimal reporting requirements, intended to cut down on paperwork and PI time use. Consideration also should be given to more direct interactions between researchers and growers or grower representatives. One method of doing this is described in the set of recommendations below.

RESEARCH COMMUNICATION AND INFORMATION EXCHANGE

The greater the number and size of research projects that are supported in the effort to control HLB, the more complex becomes the task of sharing results between investigators and fully monitoring advances. An effective HLB control strategy may arise out of the combination of results from two or more otherwise unconnected projects, whether the combination is synergistic or merely additive. Presumably the probability of finding such a happy combination should increase with the number and size of research projects, as should the number of new ideas on control strategies, providing the merits of the new combination can be recognized.

Appendix M presents a case study on the benefits of improving communications between researchers working on various aspects of the same general problem. The great benefit of co-location of researchers, as well as good communications with a wide array of researchers at other sites, may be seen from the experience with the investigation of maize stunting disease in the southern edges of the corn belt. As reported in Chapter 2, citrus research in Florida includes efforts at two major centers, the University of Florida's Citrus Research and Education Center (CREC) at Lake Alfred and the USDA-ARS US Horticultural Research Laboratory (USHRL) at Forrt Pierce, as well as efforts of smaller numbers of researchers at several other locations. The recent, marked expansion of support for research on HLB resulted in the involvement of more laboratories, on a national scale. That is, the scale of the effort that HLB is receiving and should receive is greatly in excess of possible co-location of researchers.

The experience with research projects on Pierce's disease of grapevine, and associated organizations providing various services, may be informative here. The introduction and establishment of a new insect vector, the glassy winged sharpshooter (GWSS), into southern California vineyards resulted during the 1990s in a greatly increased spread of the Pierce's disease causative agent, the Gram-negative bacterium *Xylella fastidiosa*. The resulting losses of vines and entire vineyards in southern California, and the specter of GWSS invasion of the more valuable and extensive vineyards of northern California, stimulated the initiation of several competitive research grant programs. Two of these remain, the wine-grower check-off-supported Pierce's Disease Control Program of the California Department of Food and Agriculture (CDFA) and the USDA-University of California (UC) Pierce's Disease Research Grants Program. The grant review process for these two programs is entirely integrated, with the CDFA managing the solicitation and collection of ad hoc reviews, the USDA-UC program managing the panel reviews, and a joint meeting of the advisory committees for the respective groups making the final recommendations for new and continued funding of projects by the two programs. Ad hoc and panel reviewers are recruited on a national basis, as is the solicitation of proposals.

A valuable activity of the CDFA and USDA-UC grant programs is the Pierce's Disease Symposium (http://www.cdfa.ca.gov/pdcp/Research_Symposium_Index.html), held in early December or late November each year. Participation by the Principal Investigator (PI), and preferably other research group members as well, in the Symposium is a condition of grant support from the programs. Verbal presentations are solicited, or accepted on a volunteer basis, from a subset of the PIs. The Symposium also attracts researchers who are not supported by either of the grants programs, as well as grower members of the Pierce's Disease Control Board and members of the CDFA's Pierce's Disease Research Scientific Advisory Panel. All Symposium participants are encouraged to make one or more poster presentations. Panel discussions bring together PIs who are taking similar research directions so that status of related approaches may be compared directly. Roundtable discussions are structured to encourage "brainstorming" of new approaches. Discussions at the Symposium have resulted in PIs taking new directions in their research.

Research and development communication on Pierce's disease research is also greatly facilitated through a contract from the CDFA with the University of California's Public Intellectual Property Resource for Agriculture (PIPRA). Two of the important services provided by PIPRA take the form of databases (http://www.piercesdisease.org). One of these is a nearly constantly updated repository of all references to research and other publications on Pierce's disease. The other is a compendium of progress reports submitted by research grant-supported Pierce's disease projects. The California Pierce's Disease Control Board has taken the responsibility of public education about Pierce's disease and has employed a public relations firm to prepare announcements and other documents for growers and the general public. An example is "Pierce's Disease. A Decade of Progress," which is available at http://www.cdfa.ca.gov/pdcp/.

4

Recommendations on Organizational Changes, Technology Development, and Systems Approaches for Minimizing the Impacts of Huanglongbing and Other Diseases and Pests in Florida Citrus

Given the damage that citrus greening or huanglongbing (HLB) already has inflicted on the Florida citrus industry and the threat that HLB presents to the industry's future, along with possibilities for invasions by new pests and diseases and for other challenges, a broad range of actions is recommended. The most critical needs are for immediate protection of citrus orchards against the advance of HLB, and our recommendations speak to this need. We also list other changes, which may be initiated in the near-term but are longer term in their execution, because they should result in improved disease control and crop productivity in the future.

In the context of this report, a near-term project is defined as having the potential to generate proof-of-principle or to demonstrate HLB mitigation in a period of 2 years or less. Intermediate-term and long-term projects are those with the potential to provide significant results in a time frame of 2–5 years and of more than 5 years, respectively. Our recommendations fall into the following four categories:

- Organizational changes, designated O-1 through O-5 (Table 4-1)
- Informational initiatives, designated In-1 through In-3 (Table 4-2)
- Research and technology projects with near-term and near-to-intermediate-term potential, designated NI-1 through NI-11 (Table 4-3)
- Research and technology projects with intermediate- and long-term potential, designated L-1 through L-4 (Table 4-4)

Recommendations within each of the above categories are presented in an approximate priority order. In making these recommendations, we are aware that some of them may correspond to actions that have already been initiated. We retain those recommendations for the sake of completeness, balance, and to indicate our support for such changes.

In the sections of the chapter that follow, the recommendations are described individually, by category, but because many recommendations are interrelated (within and across categories), some are discussed in relation to others and are cross-referenced in the text. Some activities are broadly enabling, and success in one area of research or effort can support the effectiveness of another activity. This reflects the nature of the overall strategy to try to achieve progress on multiple fronts and take advantage of synergies when possible.

RECOMMENDATIONS FOR ORGANIZATIONAL CHANGES

The concepts and technology of modern biology and related sciences will almost certainly be able to deliver sustainable methods for HLB mitigation not available and perhaps not even conceived at present. However, citrus production and quality must be maintained until new technologies can be put in place, or there will be no economic base to support implementing those technologies. Achieving an organized response to HLB will be a key to success in addressing the current, urgent needs of the citrus industry and developing the technology to sustain the industry in the future. Accordingly, recommendations for organizational changes are discussed first. However, the success of organizational changes is interdependent with informational initiatives and key research activities.

Recommendations O-1, In-1, In-2 and NI-1 have a high priority because of their potential for sustaining production until the to-be-developed approaches can be implemented. The committee recommends the creation of "Citrus Health Management Areas" (*Recommendation O-1*) to facilitate and coordinate control of ACP and the removal of affected trees, both in commercial orchards (*Recommendation In-1*) and in residential areas (*Recommendation In-2*). Recommendation NI-1 is intended to empower the Citrus Health Management Areas. This recommendation advocates for improved insecticide-based ACP control efforts, integrated with cultural practices, which are expected to improve management and minimize the effects on the environment and human health. Success under Recommendations NI-2 and NI-3, both concerned with achieving the detection of infected but asymptomatic trees, also has the potential to greatly improve the effectiveness of the efforts described in Recommendation O-1.

TABLE 4-1 Recommended Changes in Organizations Connected to Citrus Production

O-1. Create "Citrus Health Management Areas" in Florida.
O-2. Identify one organization and empower it to have oversight responsibility over HLB research and development efforts.
O-3. Create a centralized HLB website and data bank that is accessible to researchers and the public.
O-4. Commission an analysis of the economics of the citrus industry's responses to HLB.
O-5. Organize an enhanced annual international symposium on all aspects of HLB.

Recommendation O-1 Create "Citrus Health Management Areas" in Florida to facilitate mitigation of HLB and other threats to citrus production.

Citrus production should be preserved against the immediate threat of HLB if efforts to achieve long-term and sustainable management are to have any value. We recommend the creation of Citrus Health Management Areas to coordinate management efforts in the near term. To operate effectively, a Citrus Health Management Area should have a relatively uniform incidence of HLB, i.e. disease pressure should be relatively constant across the area. The size should be 10,000–50,000 acres. A larger area favors more effective HLB mitigation but may also require more intensive management if more growers are involved. Such areas would allow a small set of management strategies to be performed in an effective and timely fashion and their results to be evaluated. In addition, management strategies can be evaluated and compared among Citrus Health Management Areas with similar or different HLB incidences. Citrus Health Management Areas would be organized and operated by local growers and grower organizations with advice from an oversight organization (see *Recommendation O-2*). Funding for mitigation practices (i.e. insecticide applications and infected tree identification and removal) could come through grower taxes or possibly from government sources and be administered by the Florida Citrus Production Research Advisory Council (FCPRAC), the Florida Department of Citrus (FDOC) or the Citrus Research and Development Foundation (CRDF). The management areas would be charged with facilitating HLB mitigation and dealing with other threats to citrus production and quality within the area. The manager of a Citrus Health Management Areas should be empowered to enforce best management practices within the area, including cleaning-up abandoned or poorly maintained HLB-infected orchards, designing and implementing compensation plans, coordinating area-wide psyllid sprays, and reducing risk from infected urban citrus.

In some areas where incidence is low, aggressive tree removal and psyllid control will be the practice of choice. However, in areas where incidence is very high and tree removal is not feasible, only badly declining trees or blocks would be removed and those blocks with enough healthy trees to be profitable would be maintained for a few years. Such blocks would still need aggressive psyllid control to minimize spread to healthy trees or to less affected areas. In other areas, it may be necessary to remove as much citrus as possible so that new plantings can be made without the danger of being surrounded by abundant sources of inoculum. Removal of badly affected groves can result in loss of the agricultural tax exemption. Groves would need to be converted to pasture or planted with forest trees to maintain that exemption. Changes in tax law could be helpful in eliminating this impediment to removal of affected groves.

Area-wide insect control strategies should utilize “window” strategies where only certain classes of insecticides are permitted during specific periods to minimize the development of resistance (see *Recommendation NI-1*). Programs designed to train, and incentives to retain, personnel should be developed to ensure continuity of personnel familiar with needs of that region.

Research has shown that low volume aerial applications are the most convenient way to achieve Asian citrus psyllid (ACP) control in the thousands of acres of a Citrus Health Management Area. However, while aerial application for other arthropods requiring management within the area (e.g. mites) may be combined with ACP insecticides, possible effects on non-target natural enemies and other pest species must be taken into account. Care and knowledge are needed to determine which insecticides can be effectively used in low volume aerial applicators.

Control of ACP in organic orchards is challenging since there are no systemic products approved by the Organic Materials Review Institute (OMRI). This leaves products such as JMS Stylet oils and products based on neem and chrysanthemum as the only choices, and these are not likely to result in the effective long-lasting control needed to mitigate HLB. Any failure to control ACP and HLB in an orchard has direct negative impacts on surrounding orchards.

Recommendation O-2. Identify one organization, preferably an existing organization, and empower that organization to have oversight responsibility for research and development efforts intended to improve management of HLB and to plan for the future of citrus production in Florida.

It is not possible (because of diverse funding sources), and likely not desirable, to bring every funded HLB research project under the same roof. A competitive grants program should be maintained as the primary tool for enabling individual investigators to take up new approaches to HLB mitigation. However, greater coordination of projects and project funding will be beneficial, particularly as projects advance to the phase of field testing and intellectual property issues arise. Among the objectives of the proposed empowered organization would be the following: (a) Identify pre-competitive and non-competitive research goals and manage industry-sponsored research to advance those goals; this would include terminating specific projects before their ending date if appropriate; encouraging and coordinating collaborative research efforts related to these research goals at the national and international levels, including incorporating Citrus Health Response Program (CHRP) efforts into research planning; (b) Oversee the testing and comparison of new disease mitigation technologies (see *Recommendations NI-2* and *NI-3*) and provide advice in the development of Citrus Health Management Areas; (c) Manage regulatory and intellectual property, and submissions to and negotiations with regulatory agencies, related to citrus cultivars and disease control technology; (d) Provide for public education on citrus issues; (e) Assist in guiding lobbying objectives of Florida Citrus Mutual and other citrus grower organizations, and (f) Develop 5-year, 10-year, and 15-year plans for the future advancement of the industry.

The science management responsibilities of the proposed organization require expert input with a minimum of bias. It is critical that a scientific advisory board (SAB) be appointed. The majority of the SAB membership should consist of scientists who are not active citrus researchers. Among the responsibilities of the SAB should be to recommend research priorities, to review and prioritize research grant proposals, participate in an annual HLB international symposium, and to analyze research progress biyearly, including consideration of requests from PIs to change the direction or emphasis of the project. Through its review of research proposals and progress reports and its participation in the annual research symposium, the SAB will be in an ideal position to notice new developments that could contribute to synergistic interactions between research projects. The SAB should be charged with being alert to such possibilities.The SAB would make recommendations for continuation or re-direction of the project, or, in those rare instances when there seems to be little or no hope of further benefit, for termination of the project.

Recommendation O-3. Create a centralized HLB website and data bank with geographical HLB-incidence and other data, accessible to researchers and the public.

HLB is now present in several southern-tier U.S. states, Mexico, and Caribbean countries; other U.S. states have reported ACP findings, although not the HLB-associated pathogen, *Candidatus* Liberibacter asiaticus (*C*Las). The proposed website should include a database of the occurrence and distribution of the ACP and HLB in the United States and a bulletin board, which would serve as a source for information on scouting and sampling techniques, management recommendations, pesticide application technologies, summaries of recent research results, and other relevant content. The website should be accessible to researchers and the public and be linked to the CHRP and the University of Florida's Institute of Food and Agricultural Sciences (UF-IFAS) websites. The website could be modelled after other sites with similar audiences, such as the US Department of Agriculture Integrated Pest Management (IPM) Soybean Rust PIPE (Pest Information Platform for Extension and Education) at http://sbr.ipmpipe.org.

Recommendation O-4. Commission an analysis of the economics of the citrus industry's responses to HLB, including cost-benefit analysis and the potential of new technological developments.

Examples of the types of analyses that will be of value are: (a) cost-benefit analysis for different areas of Florida for current practices of HLB orchard inspection and tree removal; (b) anticipated research costs and potential impacts of new technological approaches to HLB mitigation and new methods for orchard inspection; and (c) relative cost analysis for alternative policies and orchard practices intended to minimize the impact of declining citrus quality and declining, or even collapsed, citrus production due to endemic HLB. The field of health care economics has developed sophisticated, but practical and both prospective and comparative, technology assessment models and simulations. These analytical tools could complement currently used agricultural methods and, in concert, may help guide HLB management strategies and investments.

Recommendation O-5. Organize an enhanced annual international symposium on all aspects of HLB.

There are about one hundred HLB-related active research projects in the United States. Methods, results, and concepts developed in one project will in many instances be unknown to researchers in other projects whose efforts could benefit if they did know of them. In many instances, collaborations between groups could have synergistic effects. Information transfer and the realization of the possibilities for collaboration are two of the obvious benefits of bringing researchers from different HLB projects together at an annual international research symposium. Experience shows that sometimes such contacts result in ideas for new and productive research directions not obvious to members of the separate projects. However, the benefits of gathering project researchers together can be leveraged and enhanced by also inviting prominent experts, particularly those whose accomplishments include the translation of research results into products and services. These experts, typically in fields directly or tangentially related to citrus research, would hear reports, interact with project researchers, and provide comments and suggestions. The SAB (described in *Recommendation O-2*) should be requested to prepare a report of the main advances presented at the symposium and actions that should be considered because of reported results, with the goal of accelerating the translation of research results into HLB-mitigation practices. This meeting should also be attended by the heads of the major funding agencies from Florida, Brazil, and other citrus-producing countries with major research programs on HLB. These representatives should meet and closely examine the projects funded by each to enable coordination of research efforts, avoid duplication, and enhance cooperation among investigators.

INFORMATIONAL INITIATIVES: COMMUNICATING TO THE PUBLIC AND PUBLIC OFFICIALS THE SERIOUSNESS OF HUANGLONGBING AND OTHER THREATS TO FLORIDA CITRUS PRODUCTION AND THE COUNTERMEASURES THAT MAY BE NEEDED

Most public officials who are involved in agriculture or the funding of agricultural research projects are well aware of, and reasonably knowledgeable about, the status of HLB and its implications for the citrus industry and the broader economy of Florida. However, there are individuals and groups whose actions and decisions could have an impact on HLB management but who do not have sufficient understanding of the potential long-term effects that the HLB problem could have on the economy. The need for prompt detection and removal of infected trees, and the rigor required to mount an effective HLB management program, are still not appreciated by many growers. Some homeowners are aware of the HLB problem, but do not recognize that HLB-affected trees in their landscape plantings may be contributing to the situation and that the removal of those trees could be helpful. Officials, especially at the county and city level, are often not aware of the seriousness of the problem and its implications for the economy of their area. Table 4-2 lists initiatives for communicating information about HLB to different audiences.

TABLE 4-2 Recommended Informational Initiatives

In-1. Expand extension efforts emphasizing the importance to HLB management of removing infected trees from groves.
In-2. Encourage homeowners to remove and properly dispose of backyard citrus trees, particularly HLB-affected trees.
In-3. Communicate information on HLB and its potential economic impact to government officials at the federal, state, county, and city level.

Recommendation In-1. Expand extension efforts emphasizing the importance to HLB management of removing infected trees from groves.

To the extent possible, recommendations for HLB mitigation, through inoculum removal and ACP management, should be agreed upon among researchers and extension agents and implemented uniformly in the Citrus Health Management Areas (*Recommendation O-1*). Current differences of opinion among extension agents have been detrimental to HLB management, causing confusion among growers. Programs for management of psyllids are advancing and improving rapidly, but there is a general lack of appreciation of the importance of rapid detection and removal of affected trees for mitigation of HLB. The extended latent period between infection and symptom development in citrus means that incidence may continue to rise for 2 to 3 years after a program is initiated, even if the program followed is rigorous. The early rise in incidence gives the incorrect impression that removal of infected trees is ineffective and makes it difficult to justify the several hundred dollars per acre annual cost of continuing the program.

In addition, the application of micronutrients and materials that induce systemic resistance has been promoted by some growers and extension personnel. However, there is no firm evidence that these measures do more than temporarily improve tree appearance, and it is likely that they are counter-productive because they leave sources of inoculum, in the form of symptomatic trees, in place in the grove. The use of micronutrients and systemic resistance agents should be discouraged by extension agents unless substantial evidence of the efficacy of these agents is derived from research projects with appropriate controls. Training programs by the Extension Service for HLB scouts in the detection of HLB and scouting methods would be useful in the effort to reduce *C*Las inoculum.

Recommendation In-2. Encourage homeowners to remove backyard citrus trees, particularly HLB-affected trees.

*C*Las-infected citrus in residential areas contributes significantly to the inoculum available for spread of the disease in commercial citrus. Homeowners, in general, are unaware of HLB, that their infected tree soon will be useless, or the importance to the citrus industry of removing infected trees promptly. In general, the suburban setting is not well suited to the implementation of the intensive disease control practices needed for long-term citrus culture, and homeowners should be encouraged to replace citrus with other species to reduce potential reservoirs of disease. The Extension Service should use the news media and Internet to help inform homeowners about HLB, encourage removal of affected citrus and related ornamental species such as *Murraya*, and provide information on citrus substitutes to help discourage planting of citrus in the home landscape. Information on the availability of citrus substitutes should be publicized by the Extension Service. At some point in the future, resistant varieties of citrus may be available and allow re-establishment of citrus in the home landscape.

Recommendation In-3. Communicate information on HLB and its potential economic impact to government officials at the federal, state, county, and city level.

Citrus industry leaders and the Extension Service need to do more to inform local officials about the threat posed by HLB. The citrus industry has faced serious challenges in the past that have been publicized, but the industry has always been able to deal with them. In this case, even under the best scenario, HLB is likely to change the industry permanently. Public officials will have to deal with the consequences of those impacts in terms of the local economy, employment possibilities, and reduced tax receipts.

RECOMMENDATIONS FOR RESEARCH AND TECHNOLOGY WITH THE POTENTIAL TO ADVANCE HUANGLONGBING MITIGATION IN THE NEAR-TERM OR NEAR-TO-INTERMEDIATE-TERM

The near-term efforts in HLB mitigation are focused on (a) clean-stock programs; (b) ACP suppression; and (c) reducing sources of inoculum. The use of protected environments, such as screen houses, for citrus budwood production is now accepted practice in the industry and legally mandated, so we do not comment on clean stock programs here. Table 4-3 lists recommendations for research and development that are likely to generate significant results in 2 to 5 years. Some of these, particularly NI-1 (and its subcomponents), NI-2, and NI-3 will be important to implement in synchrony with organizational changes and informational initiatives. They are aimed at improving the effectiveness of ACP suppression and inoculum reduction in the short-term. Recommendation NI-7 is also focused on ACP suppression, but will be scientifically challenging to achieve. Recommendations NI-4, NI-5, NI-6, and NI-8 through NI-11 are directed at making the investments that may generate useful novel concepts for HLB mitigation.

TABLE 4-3 Near- and Near-to-Intermediate-Term Recommendations

NI-1. Improve insecticide-based management of ACP.
NI-2. Support searches for biomarkers that may be exploited to detect *C*Las-infected citrus.
NI-3. Establish citrus orchard test plots for evaluation of new scouting and therapeutic methods.
NI-4. Accelerate the sequencing, assembly, annotation and exploitation of a sweet orange genome to provide a powerful tool for all future citrus improvement research.
NI-5. Support development of HLB model systems.
NI-6. Exploit the *C*Las genome sequence for new strategies of HLB mitigation.
NI-7. Support research aimed at developing alternative ACP management strategies.
NI-8. Support small-scale studies on the feasibility of alternative horticultural systems suited to endemic HLB.
NI-9. Support demonstration of RNA interference (RNAi) effects for possible suppression of ACP.
NI-10. Develop *in vitro* culture techniques for *C*Las to facilitate experimental manipulation of the bacterium for insights into gene function.
NI-11. Sequence, assemble and annotate the ACP genome to provide a basis for new approaches to ACP management**.**

Recommendation NI-1. Improve insecticide-based management of ACP.

Approaches recommended here, applied in concert, will support Recommendation O-1, which advocates creation of Citrus Health Management Areas in Florida to facilitate mitigation of HLB and other threats to citrus production. The suppression of ACP populations by insecticide application will be a necessary part of HLB mitigation for the foreseeable future; e.g., until sources of inoculum can be drastically reduced (*Recommendations NI-2* and *NI-3*) or *C*Las-resistant citrus (see *Recommendation L-1*) becomes available for planting. We strongly support the idea that present management strategies can be improved by gaining better understanding of ACP and *C*Las, their ecology and epidemiology, and the presently available (or soon to be available) tools that can aid in their management. Recommendation NI-1 is concerned with improving conventional insecticide suppression of ACP by integrating four types of research and development activities:

Recommendation NI-1a. Develop new methods to fine-tune field surveillance of ACP via more efficient and consistently applied trapping or other methods, to improve timing and targeting of insecticide applications.

At the moment ACP populations are monitored differently in different regions. Additional effort should be invested in developing more effective traps that will provide an accurate assessment of the ACP population so sprays can be more effectively timed. Sampling methods also should be refined within and between areas to help describe actual population trends and help in area-wide management strategies. Monitoring and analyzing ACP populations over the course of each year is also important for observing long-term trends and examing their relationship to environmental factors. Surveillance should not be limited to active orchards. Declining or abandoned orchards and citrus in urban landscapes should also be monitored to identify local ACP populations for treatment before they disperse. Well-timed applications and proper use of insecticides, based on surveillance for ACP and expected year round variations in ACP populations, present an opportunity to maximize insecticide effectiveness while minimizing risks.

Recommendation NI-1b. Evaluate new insecticides, adjuvants and application methods for their effectiveness against ACP or HLB infection.

It is critical for near- and intermediate-term mitigation of HLB to identify effective new insecticide active ingredients and adjuvants and to critically assess application techniques (soil, trunk, and aerial; low-volume, airblast, and other advanced methods) for both adult and nymphal stages of ACP. Of particular interest are insecticides with good safety profiles and with chemistries distinct from those of insecticides currently in use on citrus. Many new adjuvants have characteristics that enhance their penetration into the tree canopy and uptake in the leaves. Criteria for evaluation of application methods should include enhancement of insecticide effectiveness, ability to cover large areas quickly, compatibility with frequent HLB monitoring and low application costs. The citrus industry should foster greater collaboration and partnerships with the pesticide industry, and growers and researchers should continue to work with the federal minor-use pesticides program (also known as IR-4) and take advantage of legislation that will help evaluate the effectiveness of, and obtain needed registration data for, insecticides critical to the citrus industry. The need to conserve beneficial organisms, with due consideration to the economics of the production system, is already appreciated by pest control personnel but needs to be emphasized.

As ACP populations increase, it is likely that some insecticide resistant ACP strains will develop. There is anecdotal evidence that resistance to neonicotinoids may be starting to develop. This is troubling because neonicotinoids are being used both as a systemic drench for longer-term control on young trees and as foliar sprays. Insecticides, particularly those that have proven to cause only minimal harm to the environment and human health, must be protected from becoming obsolete because of resistance development, such as that caused by inappropriate season-long application of neonicotinoids or their use as foliar sprays. One of the advantages of the proposed Citrus Health Management Areas (*Recommendation O-1*) is that they can focus on minimizing development of insecticide-resistant ACP.

Recommendation NI-1c. Investigate the behavioral ecology and *C*Las transmission biology of ACP to improve timing and other aspects of insecticide application.

Research and practice in other systems suggests that increased understanding of insect behavioral ecology can facilitate insect management and mitigation of insect-transmitted pathogens. Research on aphids revealed the timing of dispersal flights, including mass migrations, and suggested strategies for successful monitoring of aphid populations. ACP is a new pest in Florida, and our insufficient knowledge about its interactions with its new environment limits the ability to manage ACP effectively. Near- and intermediate-term research efforts should be able to gather data on the interactions of ACP (both adults and immature forms), with *C*Las, plants, and the environment that can improve the effectiveness of insecticide usage and reveal points in the ACP life cycle that may be vulnerable to interdiction.

No definitive study has been published regarding ACP flight activity, e.g., flight range and seasonality, which would provide the ability to predict times at which adults are likely to migrate and generate information for epidemiological models. Convenient measures of macro-scale aspects of ACP behavior, such as the attractiveness of intact orchard trees for ACP are not available. Results from these studies would provide a rational basis for seeking and identifying behavior-modifying natural and synthetic products for managing ACP. At the very least, study of ACP flight behavior, sex attraction (Wenninger et al., 2008), or orientation to its host plant (Wenninger et al., 2009b) should yield data useful for improving methods to monitor psyllid movement.

Insect developmental processes and population increase are temperature driven in major part, so knowledge of local temperatures is a critical variable. Developmental and nutritional status of the host plants and climatic conditions (e.g., humidity and rainfall) are also very important. For example, flushes of new growth in citrus are important for influencing ACP flights and reproduction success. Other environmental factors may influence the timing and efficiency of long distance versus short distance spread, which can inform decisions on establishing distances between HLB-affected old orchards and replantings.

*C*Las infections may influence ACP behavior. For example, the increased carbohydrate accumulation characteristic of *C*Las-infected plant tissue may be a *C*Las adaptation mechanism that favors ACP feeding and oviposition on infected tissue and therefore enhanced acquisition of *C*Las. The circulative/propagative transmission mechanism likely to be involved in ACP-*C*Las interactions requires delivery of *C*Las into the host in saliva. Understanding salivation physiology or behavior might reveal targets for plant genetic engineering or other approaches to preventing ACP feeding.

Compared to the adults, ACP nymphs acquire *C*Las efficiently and they may be more susceptible to management efforts. Unlike adults, nymphs excrete honeydew as waxy-appearing filaments. This form of excretion most likely evolved to protect tightly clustered immatures from fouling one another with sticky honeydew. The effect of these secretions on natural enemies should be investigated. Since the waxy excretion is a waste product from ACP digestion and metabolism, analysis of this material can be used as a bioassay for compounds that have negative affects on nymph development. Detailed observations of immatures as they develop from egg stage to adult should be conducted in the field and greenhouse (e.g., by video recording). Important characteristics are the spatial dynamics of nymphs over time, excretory behavior, interactions with parasites, predators and other biotic stress, interactions, if any, with ants, and effects of rain, wind and other weather-related factors on aggregations. This information should be acquired for both a highly susceptible host (e.g., sweet orange) and a poor or marginal host (e.g., mandarin orange). Depending on results from the above, evidence of pheromones (alarm, marking), acoustic signals or other stimuli may be obtained.

Additional knowledge is needed about the ability of ACP to acquire and inoculate *C*Las. For example, how efficient can asymptomatic infected trees be for *C*Las acquisition by ACP? Orchard test plots including such trees (*Recommendation NI-3*) could be an experimental tool for answering this question. Some reports suggest that the latent period between *C*Las acquisition and inoculation by ACP may be as short as 8 days or as long as 25 days. Is the time between ACP acquisition of CLas and becoming inoculative really highly variable, or can it be predicted based on environmental conditions? The spread of HLB may be greatly reduced if insecticides can be applied to the ACP population prior to it becoming inoculative and prior to its movement to a new host.

Although the existence of unrecognized reservoirs of *C*Las inoculum has been postulated, the spread and accumulation of HLB would seem to be accounted for by infected ACP and citrus species. Therefore, requests for support of research on other reservoirs should be considered only if they are based on preliminary evidence that such reservoirs could be of epidemiological significance.

As more information on ACP behavioral ecology and *C*Las acquisition and retention in the vector is accumulated, incorporation of the data into a mathematical model may allow prediction of ACP movement patterns. This will be a longer term effort (see *Recommendation L-3*).

Recommendation NI-1d. Evaluate new cultural practices for their effectiveness against ACP or new HLB infections.

A list of practices that can be used to reduce ACP and HLB is included in the 2010 Citrus Pest Management Guide (Rogers et al., 2010). These include practices that reduce the occurrence of new growth flushes and treatment of infected trees with a quick-acting insecticide to reduce the potential for ACP to move to other hosts before the trees are removed. These practices should be augmented with new practices where appropriate, be implemented over an entire Citrus Management Health Area, and be evaluated for effectiveness.

Discussion of the Merits of NI-1

Favoring rationale: In Florida, there is considerable existing expertise on insect management, the management of other pests, insect ecology, and the modification of cultural practices, such as management of flush. There are many insecticides registered and recommended for ACP in Florida (Rogers et al., 2010), and this situation is favorable for development of effective IPM programs for ACP in Florida citrus. Florida entomologists are generally prepared to collect the types of information described in Recommendation NI-1c on adult and immature forms of ACP and the interactions of ACP, *C*Las, citrus trees and the environment. The ACP immature stages are especially under studied. Since late stage ACP nymphs are far more efficient at acquiring the HLB bacterium than adults, it is logical to look closely at their development, ecology and behavior with an eye towards interfering with nymph development. Since the immatures may be more readily manipulated than adults, they could be more vulnerable to management practices. Better integration of research and application efforts can produce a whole that is greater than the aggregate of its parts.

Disfavoring rationale: It is not apparent that there is a sufficient level of cooperative effort between research groups to achieve the goals of this recommendation. Additionally, the occurrence of citrus trees whose owners choose not to apply effective insecticides is a significant impediment to effective ACP management. Examples are abandoned and semi-abandoned groves and backyard ornamental citrus as well as citrus in organic production. Any failure to control ACP in a given setting will likely have direct negative impacts on surrounding commercial orchards. Vigorous ACP suppression programs may include applications of aldicarb, a very effective insecticide but one that is of concern to human health and to the environment.

Examples of currently or recently supported research projects and relevant results: There are several currently funded projects that address insecticidal practices for ACP. These include at least two projects investigating ultra-low volume techniques for applying foliar insecticides over a wide area quickly, three projects investigating different rates and different insecticides for seasonal control of ACP, one project investigating the use of citrus flushes and dormant spray oils for ACP control, and another investigating cross-resistance between insecticides and the potential for evolution of resistance.

Examples of possible future research projects: It is likely that significant benefits to management of ACP will arise from technologies that: (a) help time insecticide treatments through the use of ACP monitoring tools; (b) apply the insecticides quickly over large areas and utilize techniques that ensure effective coverage; (c) deliver insecticides that provide maximum effectiveness against ACP with maximum safety to applicators, consumers and the environment; (d) minimize the development of resistance to insecticides; (e) conserve beneficial organisms within the citrus agroecosystem; and (f) are adaptable to application under Recommendation O-1 to the Citrus Health Management Areas. Registration of insecticides that are effective when applied with low volume applications will be crucial to ACP management since this will allow large areas to be treated rapidly when needed. Alternative insecticides with equivalent efficacy to aldicarb are urgently needed. It is important that results from research on insecticide management of ACP be conveyed through extension services and private consultants. This would be best achieved by involving them directly in the research trials so they are full partners.

Suggested support mechanism: Projects in support of alternative management strategies should be funded by both Florida programs and USDA programs such as Pest Management Alternatives Program (PMAP) and IR-4. It is reasonable to expect insecticide manufacturers to also provide support for such projects.

Time to outcome: Based on the experience of the Brazilian citrus industry and in one area of Florida, investments in improved insecticide applications have great potential for near-term, as well as intermediate-term, benefits to ACP management.

Other sections of this report containing related information: Chapter 2, pp. 34–37 (transmission by ACP); pp. 47–49, (ACP biocontrol and guava as ACP repellent); pp. 53–55 (insecticidal control); Chapter 3, pp. 72–77 (reducing ACP access to citrus); Appendix J; Appendix K (insecticide and spray effects on ACP mortality).

Recommendation NI-2. Support searches for biomarkers that may be exploited to more efficiently detect *C*Las-infected citrus, with emphasis on identification of asymptomatic infected trees.

Visual surveillance by trained personnel is now the only reliable and cost effective method for identifying potential reservoirs for HLB, both in and out of the orchard. Trained scouts can detect symptoms of HLB-infected trees with reliability, but typically not until 6–18 months or possibly years after the infection of citrus with *C*Las by ACP and the tree has become a potential reservoir for spread of HLB. Automation of the detection of symptomatic infections would be of great value (e.g., in allowing increased frequency of scouting at a lower cost). Compared to a symptomatic tree, a *C*Las-infected asymptomatic tree on average is (a) available as a *C*Las source for fewer months; and (b) has a lower *C*Las titer and proportion of the tree invaded by *C*Las. Therefore, the ability to detect *C*Las infections at reasonable cost in *asymptomatic* trees could have a revolutionary effect on HLB mitigation practice and effectiveness, especially, but not only, in areas of low HLB incidence with large plantings of citrus that are away from urban areas or poorly maintained/abandoned groves. ACP suppression efforts might be relaxed to some extent if *C*Las reservoirs could be more effectively eliminated.

Identifying *C*Las-infected orchard citrus trees may rely on detecting *C*Las itself or on observing the signal of an infection-related altered-state signal of the tree, a biomarker, that would telegraph the presence of infecting *C*Las. Highly sensitive and specific means for detecting *C*Las are available. However, *C*Las is unevenly, highly locally, and unpredictably distributed in an asymptomatic tree (Tatineni et al., 2008), creating an unmanageable sampling problem for detection. Therefore, finding asymptomatic infected trees by *C*Las detection will require new information on the patterns of infection within trees. Tree cytological and physiological changes (Etxeberria et al., 2009; Folimonova et al., 2009) not suitable for use as biomarkers directly nevertheless may enhance our understanding of *C*Las spread in the tree, guiding sampling for *C*Las detection and the development of new biomarkers.

The spatial and temporal aspects of *C*Las invasion of the tree and their relationship to the spatial and temporal development of biomarkers, especially prior to appearance of symptoms, will be an important basic component of research advancing this recommendation. How is the movement of *C*Las in the tree related to symptom development and the activation of relevant biomarkers? Are there cytological changes such as phloem necrosis which, though not useful as an easily assessed biomarker, can be used to trace the movement of *C*Las? Results from investigations related to these questions could enhance sampling procedures for more efficient detection of asymptomatic, infected trees.

*C*Las-infection specific biomarkers, should they be discovered, have the potential to be detected in parts of the tree not reached by the *C*Las infection, thus increasing the probability of detection. Three classes of potential *C*Las infection-revealing biomarkers are volatile organic compounds (VOCs) emitted by the tree, optical changes in the tree, and changes in the concentrations of small molecules or of proteins, nucleic acids or other macromolecules in the tree tissue. Only the rare biomarker provides a strong and unequivocal signal; typically the detection requires instruments of high sensitivity, high specificity, and ease in use.

VOC biomarkers: The potential of VOC measurements to detect plants entering a state of biotic or abiotic stress is widely recognized. VOC detection in the parts-per-trillion range probably will be required, a sensitivity that has been achieved by dogs and bees and, recently, by the relatively new differential mobility spectrometry (DMS) instruments (Krebs et al., 2005; Molina et al., 2008; Zhao et al., 2009). In addition to direct searches for VOC biomarkers, analysis of changes in gene expression as revealed by DNA arrays, or gene chips, can be interpreted using state-of-the-art bioinformatics procedures to discover infection-specific alterations in metabolic networks that are predictive of changes in proteins and small molecules, including tree volatiles.

Optical biomarkers: Reflectance measurements, and more generally the full range of biophotonics techniques, have the potential to distinguish uninfected from infected trees, even trees that appear not to be infected. Even an optical scanning method of limited capabilities could be of value; for example, a method that can detect infected trees with a significant rate of Type I (false positive) errors (e.g., due to non-HLB disease conditions), but that has a negligible rate of Type II (false negative) errors. Application of such a method would be followed by targeted visual inspection or PCR analysis rather than a full tree-by-tree survey.

Chemical biomarkers: In several systems, induction of plant innate immune response has been reported to occur early in the infection process, before the appearance of symptoms, and was detectable locally as well as at more distant points in the plant (Boller and Felix, 2009). Whether any pre-symptomatic biomarker spreads uniformly or to specific locations in citrus trees is not known but can be readily investigated. The number of trees, and the probable requirement for multiple samples per tree, eliminate traditional wet chemical methods as viable candidates for biomarker detection. However, chemical sensing electrodes and chemically reactive "dip sticks" could eliminate or minimize extraction procedures.

One often proposed approach to *C*Las-infection detection is the planting and surveillance of "sentinel" plants. Compared to citrus, a sentinel plant would need to be more attractive to ACP and show symptoms of *C*Las infection sooner. *C*Las has been detected in individual ACP collected in orchards that were without visibly infected trees (Manjunath et al., 2008). Technology that will probably detect *C*Las-infected but asymptomatic orchard citrus trees or *C*Las-infected ACP will be more effective than a sentinel plant at revealing the presence of HLB, especially since a requisite sentinel plant species has not been identified.

Discussion of the Merits of NI-2

Favoring rationale: In-orchard detection of biomarkers as tree volatiles, by optical methods, or by non-traditional wet chemical methods has the potential to augment and revise current scouting practices and allow more efficient and frequent surveillance to achieve unprecedented reductions in *C*Las reservoirs. Recent improvements in the sensitivity, specificity, portability and ease of use of instruments suited to biomarker detection favor a look at new detection technologies at this time.

Disfavoring rationale: Although DMS and other sensitive instruments for detecting VOCs have been developed and some have been significantly miniaturized, none are suited at this time for direct in-orchard detection of trees with altered VOC emissions. Increases in the quantity of VOCs released by the biologically stressed plant are more common than the production of new VOCs, which would be more easily detected than quantitative changes. The only high-sensitivity, high-specificity method for detecting *C*Las infections in asymptomatic trees is by conventional PCR analysis, which currently would be impossible to apply on an orchard scale for the 50 million citrus trees in Florida. It does not appear that any optical measurement has been able to distinguish HLB from other citrus maladies. Differences in chlorophyll content appear to be responsible for the most prominent changes in spectral reflectance of leaves from HLB and healthy citrus. Changes in chlorophyll content may have a variety of causes not related to HLB. Although experiments with greenhouse-grown citrus trees suggest that biomarkers of asymptomatic *C*Las infection have been observed, orchard citrus trees are very large compared to experimental greenhouse specimens and are subject to a greater range of biotic and abiotic stress, so biomarkers discovered in greenhouse experiments may not be relevant to orchard conditions.

Example of currently or recently supported research projects and relevant results: Research projects in which gene expression, protein accumulation, or metabolite composition are compared for *C*Las-infected and non-infected citrus are in progress. All have the potential to reveal new *C*Las infection biomarkers. References cited above identify projects aimed at discovering VOC and optical biomarkers.

Examples of possible future research projects: Although not a research project, if the orchard test plots described in Recommendation NI-3 are created and made available to researchers, it will be possible to rapidly evaluate biomarkers of all three types described here as well as any instrumentation designed to exploit those biomarkers.

Suggested support mechanism: It is possible that the detectors and software needed for discovery of *C*Las-infected citrus trees using optical or VOC biomarkers already are available in a university, institute or government laboratory or at a commercial firm. The offering of an inducement prize may activate the shortest path to deployment of *C*Las-infection-detecting equipment, with the Recommendation NI-3 orchard test plots serving as the testing ground for demonstrating successful detection. Competitive grants funding should be continued in support of biomarker discovery.

Time to outcome: Near- to mid-term for discovery of suitable biomarkers, mid-term for development and deployment of instrumentation for improved HLB scouting.

Other sections of this report containing related information: Chapter 3, pp. 69-71 (removing HLB-affected trees).

Recommendation NI-3. Establish citrus orchard test plots that include *C*Las-infected but asymptomatic trees, as well as symptomatic trees, for evaluation of new scouting methods and therapeutics.

The establishment of test plots is intended to provide a systematic approach for realistic evaluations of methods for early detection of *C*Las-infected trees, as described in Recommendation NI-2, and for evaluating the efficacy of potential therapeutic treatments. It is crucial that new scouting methods and new therapeutics, already developed to the point of showing promise and perhaps proof of concept under laboratory or greenhouse conditions, be examined under orchard conditions with suitable controls before further investments of time and money are made. As an example of the need for this information, a therapeutic treatment that improves the appearance of trees but fails to reduce *C*Las titers can have serious consequences because *C*Las reservoirs would be maintained rather than removed.

Test plots could be established and maintained under contract and with cooperation from growers or owners. A plot may, for example, be located in an isolated, abandoned grove. Each test plot should be in a different region or environment, so that the general effectiveness of therapeutic agents can be assessed and biomarkers specific for *C*Las infection can be validated to be context-independent. Each plot should have trees documented to be (a) infected and showing HLB symptoms; (b) most importantly, *C*Las-infected but asymptomatic; and (c) uninfected, to the extent that freedom from *C*Las can be verified.

Identifying infected but asymptomatic trees probably will require research effort and may involve extensive sample collection from suspect trees and sensitive and high-throughput PCR analysis. Alternatively, a surrogate for an asymptomatic tree might be prepared by pruning out symptomatic branches. Less realistic, but possibly useful for preliminary tests, would be artificially inoculated trees in the greenhouse or in the orchard. Investigators may be able to find other ways to apply current, and likely arduous, methods to identify a small number of infected but asymptomatic trees in existing citrus orchards.

Over time, asymptomatic trees will show symptoms, and uninfected trees will become *C*Las-infected. Therefore, assessments of infection state should be carried out periodically, and a database recording the infection state of trees in the test plots will need to be updated accordingly. Plots should be made available for use at cost to qualified and responsible investigators and competitors for inducement prizes (see *Recommendation NI-2*, Suggested Support Mechanism). The infection state of specific trees would be confidential information to be revealed only after scoring by investigators and competitors have been completed.

Discussion of the Merits of NI-3

Favoring rationale: It is critical and urgent to determine whether compounds and mixtures considered to have therapeutic activity and biomarkers intended to reveal early *C*Las infection are actually effective and to do so at a minimum of expense and with convincing outcomes. Only a systematic approach with centralized test sites can accomplish this.

Disfavoring rationale: Although the accumulation of *C*Las has been assessed in infected, symptomatic orchard citrus trees (Li et al., 2009; Trivedi et al., 2009), the distribution of *C*Las in trees at early stages of infection is undocumented. In the absence of such documentation, large numbers of candidate trees may be required in order to assure the presence in the sample of a sufficient number of infected but pre-symptomatic trees, significantly increasing the costs of creating and maintaining the reference plots. Whether information on, and statistical analyses of, the spread of HLB in groves will be sufficient to assist in the design of the reference orchard is unknown. The establishment of HLB reference plots will be antithetical to HLB mitigation practices in many areas, limiting the choices of available plots.

Example of currently or recently supported research projects and relevant results: None known.

Examples of possible future research projects: The HLB reference citrus orchard would be a service to researchers and technologists rather than a research project.

Related information and references: As indicated in Chapter 3, the average titer of *C*Las in infected but asymptomatic trees is lower than it is in symptomatic trees, and, under greenhouse conditions, the *C*Las titer may be more than 10^4 greater in a symptomatic tree than an asymptomatic tree at an early stage of infection. Where the titer is still very low, the tree should have a significantly diminished ability to serve as an HLB source compared to symptomatic trees.

Suggested support mechanism: Creation, assessment and maintenance of HLB reference citrus orchard plots should be under the control of a central authority, presumably under supervision of a contractor or a research organization. Detection of *C*Las-infected asymptomatic trees should be by certified methods and be subject to review by state or federal authority because the specification of "infected" could be subject to contention, especially where an inducement prize is at stake.

Time to outcome: Establishing HLB reference citrus orchard plots should be initiated as soon as possible because there is a critical need for surveillance technology capable of more rapidly and efficiently detecting symptomatic trees than current scouting practices allow, and facile detection of infected but asymptomatic trees would revolutionize HLB mitigation practices.

Other sections of this report containing related information: Chapter 2, pp. 49–50 (survey methods), and pp. 67–69 (removing HLB-affected trees).

Recommendation NI-4. Accelerate the sequencing, assembly, annotation and exploitation of the sweet orange genome, to provide a powerful tool for all future citrus improvement research.

The International Citrus Genome Consortium (ICGC), including groups from the United States, Brazil, France, Spain, and Italy, was established in 2003 to sequence a citrus genome and develop genomic tools to support citrus research worldwide (http://citrus_static.hivip.org/reports/2009/08/04/FCPRAC_ICGC_quarterly_report_8-09.pdf). This consortium has generated many publicly available expressed sequence tags (EST) libraries, which collectively have over 550,000 ESTs (http://www.citrusgenome.ucr.edu).

In 2007, a 1.2X-coverage genome sequence of sweet orange was released by the US Department of Energy Joint Genome Institute (Talon and Gmitter, Jr., 2008). Using next-generation sequencing (454 of Roche Diagnostics), the ICGC has now reached over 30X coverage of the sweet orange genome coverage (FCPRAC, 2010). In addition, a number of citrus Bacteria Artificial Chromosome (BAC) libraries have been produced and are being curated at the Clemson University Genomics Institute. This effort should be redirected to take advantage of current technology and expanded to provide the underpinnings of a sweet orange conventional breeding system.

Discussion of the Merits of NI-4

Favoring rationale: Having the genome sequence of an organism in hand provides a tool with often unanticipated applications. Knowledge of the genome can be applied to citrus genetic improvement in general. Deciphering the citrus genome and additional genomic information (e.g., single nucleotide polymorphisms (SNPs) and ESTs) will provide scientists with a number of genomic tools that can be used to understand the genomic response of citrus in response to HLB, bacterial infection, and stress. Genomic-based research tools can support the creation of differential gene expression profiles to highlight differences between healthy and diseased citrus to aid in discovery of biomarkers applicable to both detection of diseased trees (*Recommendation NI-2*) and DNA markers for plant breeding. Genomic information and tools can support biotechnology and conventional approaches to support disease resistance in citrus. An example is knowledge of promoters and targeting sequences in support of transgenic citrus work. Progress on other plant genome projects suggests that progress on the citrus genome, and development of associated genetic tools, can be accelerated.

Sequencing of plant genomes, such as tomato and potato, have historically been conducted through large international consortia whose members can share the high costs and extensive computational investments and can manage a large-scale undertaking. The International Solanaceae Genome Project consortium involves 10 countries. By 2009, six years after the initiative was launched, one-third of the tomato genome had been sequenced. The Cacao Sequence project, an initiative launched in 2008, plans to sequence the cacao genome in under 5 years with sponsorship primarily from Mars, Inc., the USDA, and the participation of a few strategic partners. Declining sequencing costs and improvements in computational capacities may not warrant complex international collaborations for future sequencing projects. A global consortium may, in fact, delay the decision-making process and slow the pace of sequencing.

Early in 2009, a cucumber-sequencing project was begun by a group at the Beijing Genome Institute-Shenzhen with collaborators in several countries. This project employed a combination of traditional Sanger sequencing, which provides reads of ~700 base pairs (bp) and a state-of-the-art very high-throughput method (reads of 50 bp) in a hierarchical shotgun sequencing strategy to obtain a total of 26.5 billion bp of sequence. The sequencing effort produced 72-fold coverage, and 97.5 percent of the assembled and annotated sequence had a coverage of 10X or greater. The results revealed 26,682 genes and were published online in early November 2009 (Huang et al., 2009). The cucumber genome is 367 megabase pairs (Mbp); the sweet orange genome is 382 Mbp.

The accelerated effort recommended here could complement the efforts of the existing ICGC, as well as use the results and libraries it has generated. However, a new organizational and funding structure, and possibly new partners to rapidly complete the citrus genome sequence are needed. The proposed strategy is to identify a small genome steering committee that represents the most important stakeholders and technology providers and a single funding source to complete the genome sequence. The steering committee needs to be equipped to respond rapidly to evolving sequencing technologies and assembly strategies should take into account complications introduced by citrus heterozygosity.

The overall project should develop high-density SNP markers and the assay platforms that will enable citrus breeders and scientists to accelerate genetic improvement strategies. The goal is to create an integrated project designed to complete, in short order, (1) the sequencing, assembly and annotation of a sweet orange genome using contemporary methods and meeting contemporary quality standards; (2) apply re-sequencing to document genomes of other citrus, including wild species; and (3) apply the information gained from genomic analyses to establish a workable conventional breeding system for sweet orange, including facile identification and evaluation of cross progeny.

Disfavoring rationale: The ICGC already consists of participants from several countries. If this recommendation is implemented, great care will be needed to ensure that the pace of sequencing the citrus genome is accelerated without the unintended effect of adding another layer of complexity to the existing organization of this initiative or reducing the enthusiasm of present participants. The estimated cost of sequencing the sweet orange genome is a few million dollars.

Example of currently or recently supported research projects and relevant results: The ICGC already is in progress.

Examples of possible future research projects: The end result of citrus sequencing efforts would be a large and very useful body of new information: the identities, structures, and in many instances the functions of the genes of sweet orange and eventually other citrus species and varieties. An example of the useful information expected would be a list of candidate phloem proteins (Huang et al., 2009), which would, in part, define the medium in which the phloem-dwelling *C*Las replicates in the citrus tree.

Related information and references: The genome sequences of about 7 plant species have been published. The general trend for each succeeding sequence has been to substantially lower costs and shorter time to completion. Except for the complication of heterozygosity of commercial citrus genomes, there is no reason that this trend would not benefit a citrus genome project.

Suggested support mechanism: Currently, the citrus genome is being conducted through an international consortium. The goal of this recommendation is to accelerate the genome sequencing, which may require a more effective and streamlined organizational structure.

Time to outcome: Finalizing the citrus genome is a high priority for research with significant benefits to the citrus industry; an aggressive short term strategy should be put in place as soon as possible to complete the sweet orange genome in less than 2 years.

Other sections of this report containing related information: Chapter 3, pp. 71–72 (citrus transcriptome), and Appendix J (citrus genome sequencing).

Recommendation NI-5. Support development of model systems intended to reveal new approaches to HLB mitigation.

None of the elements of HLB are very amenable to controlled investigations of the underlying mechanisms of disease spread and development: not the disease agent, not the vector, not the host, and not the environment. For these reasons, we are recommending that research proposals based on model systems rather than HLB-affected citrus itself receive serious consideration. Often research in model systems can yield new insights at relatively low cost andcan allow the application of high-throughput methods, not applicable to the original system, to test many compounds or genes for their potential as HLB mitigation agents.

Discussion of the Merits of NI-5

Favoring rationale: Model systems have allowed studies to be short-term and high-throughput, saving time and money. For example, it is conceivable that a citrus rootstock could be transformed to produce an anti-*C*Las protein that would move into and protect the scion. Candidate anti-*C*Las proteins almost certainly would be tested in a model plant system before moving to citrus. Model systems provide an opportunity to create a genuinely new and effective therapeutic—a silver bullet for HLB mitigation (see *Recommendation L-2).*

Disfavoring rationale: There are likely to be only a few model systems or test beds that will be relevant to HLB control.

Examples of currently or recently supported research projects and relevant results: A Rapid Screening Process for Chemical Control of Huanglongbing is a project being conducted by C. Powell, University of Florida. According to the proposal abstract: "This involves growing *Can*. Liberibacter-infected citrus and periwinkle in culture, and adding antibacterial molecules to the culture medium and assessing the effect on the bacteria by qPCR. This will allow testing hundreds of antibacterial compounds in a short period of time, and those compounds that are effective on HLB-suppression without phytotoxicity will be selected for field trial."

Use of the tomato/potato psyllid, *Bactericera cockerelli*, vector for the zebra chip disease of potato, is also a valuable model system, in place of citrus trees and ACP. An herbaceous host has clear cultural advantages over perennial citrus and allows the use of virus vectors for high throughput testing of putative anti-*C*Las and anti-ACP genetic constructions. One of several projects underway is being conducted by B. Falk, University of California at Davis, who is developing anti-psyllid RNA interference (RNAi) (*Recommendation NI-9*) technology using tomato seedlings and *B. cockerelli*.

Example of possible future research projects: Chemical libraries could be screened for compounds capable of causing citrus to repel ACP. For example, genetically homogenous tomato seeds could be germinated in multi-well plates and then exposed to the compounds (a different chemical added to each well) in low micromolar concentrations. The treated seedlings could then be exposed to the tomato/potato psyllid and observed to see which are avoided by (or that attract) the psyllid.

Related information and references: In one study, Arabidopsis seedlings in liquid medium in 96-well plates were exposed to and subsequently infected by *Pseudomonas syringae* bacteria that induced bleaching of the cotyledons. Seedlings were exposed to micromolar concentrations of about 200 compounds from a chemical library prior to inoculation by *P. syringae*. A few sulfanilamide compounds dramatically reduced *P. syringae*-induced cotyledon bleaching and one compound reduced the *P. syringae* titer to about 10 percent of control value (Schreiber et al., 2008).

In another study, tomato seedling cotyledons were transformed with *Agrobacterium rhizogenes* to introduce an expression library of tomato cDNAs. The resulting library of tens of thousands of hairy roots was exposed to the apoptosis-inducing mycotoxin fumonisin B1. A few of the hairy roots remained white and growing. After several transfers of root segments in fumonisin B1-containing medium, the cloned and selected cDNA sequences were recovered by PCR (Harvey et al., 2008). Subsequently, the corresponding transgenes have demonstrated significant sparing effects against invasion by specific apoptosis-inducing pathogens.

Suggested support mechanism: Proposals on model systems require expert evaluation both for the feasibility of the model system and for its appropriateness to the HLB problem, favoring a competitive grants approach for providing research support.

Time to outcome: Mid-term to long-term.

Other sections of this report containing related information: Chapter 3, p. 87 (model systems), and Appendix J (research on model systems).

Recommendation NI-6. Exploit the *C*Las genome sequence for new strategies of HLB mitigation.

The *C*Las genome sequence was recently completed and annotated (Duan et al., 2009; Tyler et al., 2009). Implementation of recommendation NI-6 is intended to identify *C*Las targets for future HLB mitigation technologies.

Discussion of the Merits of NI-6

Favoring rationale: Bioinformatics can produce what is, in effect, a highly educated guess (annotation) about the functions of an individual gene from the nucleotide sequence of the gene alone. The available annotated *C*Las genomic sequence (Duan et al., 2009) revealed the presence of genes encoding several Type I secretion systems but no Type III secretion system and no cell-wall degrading enzymes. Knowing what genes are not present is as informative as the observed gene complement; for example, for use in the design of synthetic growth media for *C*Las or for insight into the offensive and defensive tactics that may be taken by *C*Las in its plant or insect host. Transfer of *C*Las genes to *Escherichia coli* or other bacterium suited to experimental manipulation or transient expression of *C*Las genes by means of bacterial- or viral- based molecular vectors in plant or insect hosts can reveal the functions of *C*Las genes. Knowing the functions of specific *C*Las genes can direct approaches to interfering with essential gene function, in effect controlling the bacterium.

Disfavoring rationale: Although the annotated *C*Las genome sequence is a valuable information source for identifying gene products as potential targets, developing and applying test systems to verify gene product function and susceptibility of the potential target to interference are more difficult and expensive.

Example of currently or recently supported research projects and relevant results: Several research groups are investigating various *C*Las genes for gene products that may be susceptible to interdiction by chemical or genetic approaches.

Suggested support mechanism: A peer-review-based competitive grants program would be the best mechanism for identifying projects likely to advance mitigation of HLB.

Time to outcome: Demonstration of proof of principle, (e.g., by reducing *C*Las growth in a model system) could be achieved in months or a few years. The actual application to citrus would be a longer-term process.

Examples of possible future research projects: The CRDF published a request for proposals intended "to test new methods of disease control made possible using the bacterial genome sequence recently published." It is likely that awards for new projects in this area will be made in early 2010.

Other sections of this report containing related information: Chapter 3, p. 68 (*C*Las genomics).

Recommendation NI-7. Develop alternative ACP management strategies.

As is widely recognized, thus far only the application of conventional insecticides has succeeded in reducing ACP populations to the point that HLB mitigation is demonstrated. However, dependence on traditional insecticides has potentially adverse consequences to sustainable orchard practice. In the future, ACP-resistant commercial citrus lines created using RNA inteference (RNAi) or other genetic means (see *Recommendations NI-9* and *L-1*) have very good potential to provide HLB mitigation. However, in the near and intermediate terms there will be a need not only for new chemical insecticides, adjuvants, and better surveillance and application methods and modified cultural practices (*Recommendation NI-1*), but also for new biochemical methods of suppressing ACP populations. Non-insecticidal ACP life-cycle disrupters and chemical and biological repellents and/or attractants have the potential to suppress ACP populations or *C*Las transmission while having minimal effects on natural enemies. ACP is the target of some natural enemies in Florida. It is worthwhile to use ACP control options that tend to preserve ACP enemies. However, for systems such as HLB, in which the insect vector transmits a pathogen which causes rapid crop loss, biological control of the insect vector alone has not resulted in significant crop protection. Therefore, we believe that at this time it will be prudent not to direct significant resources to ACP biological control strategies but rather to opportunities for other non-insecticidal chemical management.

Recommendation NI-7a. Investigate ACP-citrus interactions to develop non-insecticidal and possibly highly ACP-specific compounds capable of interfering with the ACP life cycle or its interaction with *C*las.

ACP is well adapted to and completes its life cycle on citrus, which in general ACP prefers to other plant hosts (Halbert and Manjunath, 2004). This adaptation suggests that ACP responds to specific characteristics of citrus such as plant compounds that act as ovipositional and feeding cues. Similarly, the intimate contact between ACP and citrus during feeding may involve ACP countermeasures against defenses that citrus would be expected to mount against invading insects. Results derived from research under Recommendation NI-2 (test plots) and NI-11 (the ACP genome sequence) could inform a search for compounds involved in chemical communications between ACP and citrus, and for compounds capable of interfering with such communications, e.g., with the ability of ACP to locate and lay eggs on suitable sites on the host or to protect itself against citrus insect defense. Other strategies could be aimed at discovering compounds that disrupt processes of ACP development, mating, and ability to transmit the bacterium. Obviously, compounds of the types described here, although much more difficult to identify than conventional insecticidal compounds, have the potential to be far more ACP-specific and therefore may prove more sustainable in their use than conventional insecticides.

Recommendation NI-7b. Develop repellents/deterrents and attractants for ACP.

If an appropriate system with a highly attractive plant species can be developed, trap cropping could be used to lure ACP away from orchard citrus trees and concentrate them onto plant species that can withstand an infestation and allow large numbers of ACP to be killed with minimal insecticide application. A few very successful trap crop programs have been implemented on a commercial scale (Shelton and Badenes-Perez, 2006). However, because the impact of ACP is by its transmission of *C*Las, achieving effective disease mitigation through use of repellents and attractants is far more difficult for HLB. If repellent/deterrent chemicals are identified, it is possible that they could be applied to citrus to drive ACP away. Likewise, if attractants are applied to other plant species that are not suitable hosts of ACP or HLB, they could be used to protect citrus.

Discussion of the Merits of NI-7

Favoring rationale: Reduction in the use of conventional pesticides continues to be an important trend in modern agriculture, which runs counter to continued heavy reliance on traditional insecticides for management of ACP. The reasons for this trend are several, including reduction in production costs for the grower, benefits to the environment, real and presumed hazards of pesticides to consumers and farm workers, and marketing strategies (e.g., organic food and bio-based labeling). In cases where production practices do not allow the use of traditional insecticides (e.g., organic production), alternative strategies will be especially important. The current strategy for management of ACP relies on an aggressive program of soil- and foliar-applied insecticides. History has taught entomologists that sole reliance on insecticide-based strategies will not be sustainable in the long run, or oftentimes not even in the short run. Other management strategies should be pursued as part of an overall integrated pest management (IPM) approach. Although little is presently known about specific interactions among ACP, *C*Las, the plant host and the environment, results from other vector systems suggests that information on these topics could assist in HLB mitigation.

Disfavoring rationale: Many, if not all, alternative chemical strategies for ACP management are only in the conceptual stage or in very early phases of development. There does not appear to be any alternative strategy that is ready for implementation at this time and can match the effectiveness of current insecticide application regimes. This observation suggests that the search for such strategies will not be easy. With the current HLB crisis facing the citrus industry, it would be unwise to move rapidly from reliance on traditional insecticides to approaches with incompletely explored capabilities and potential problems.

Examples of currently or recently supported research projects and relevant results: There are many currently funded projects exploring alternative management practices for ACP. These include three projects using RNAi (which are likely to be long term to application), one project investigating the ACP transcriptome, three projects using some form of trap cropping to disrupt the ability of ACP to colonize citrus, and other projects that are focusing on enhancing biological control of ACP.

Examples of possible future research projects: New strategies may arise from an understanding of the ACP genome (see *Recommendation NI-11*).

Related information and references: Whatever strategies are used to manage ACP, they should be used in conjunction with a more complete understanding of the biology/ecology of ACP as well as the landscape in which the strategies are deployed to delay the evolution of resistance to a particular tactic (e.g., Storer et al., 2001). In a perennial cropping system such as citrus, which incurs a large initial investment, durability of the system is essential for longer term sustainability. A region-wise set of management strategies (*Recommendation O-1)* is vital to the success of such a program.

Suggested support mechanism: Projects currently testing alternative management strategies should be continued and new projects should be supported on a competitive basis with proposals evaluated on scientific merit and potential benefit.

Time to outcome: Research in progress at this time may yield replacements or partial substitutes for current insecticide applications, but it is more likely that effective alternative ACP suppression approaches will be available only after years of research and development. However, incorporation of traditional insecticides into more effective and sustainable IPM programs offers the possibility of near-term improvements with long-term benefits.

Other sections of this report containing related information: Chapter 3, pp. 72–76 (reducing ACP access to citrus).

Recommendation NI-8. Support small-scale studies on the feasibility of alternative horticultural systems that may lead to citrus production systems suited to endemic HLB.

If shortages drive prices higher, some cultural practices now deemed economically unfeasible may become viable. Recommendation NI-8 is intended to encourage the exploration of new citrus production systems such as high-density plantings and screen houses. The advanced citrus production systems may be able to compress and enhance the citrus production cycle so that economic return can be realized in fewer years (Hutton and Cullis, 1981). Early economic fruit production could be reached in as little as three years, thus achieving an early return on investment.

Discussion of the Merits of NI-8

Favoring rationale: Most advanced production systems involve high-density plantings and intensive management. The advantages of these systems are that production begins earlier in the life of the grove, yields are higher, and fruit from shorter trees would be easier to harvest. Grove life would be shorter, but close spacings would allow cost recovery and profitable production before HLB eventually took over the grove, which would then be replaced.

Disfavoring rationale: The disadvantages of advanced production systems are the high initial cost of orchard establishment and the more intense management required. These systems can probably function, but the economic viability of the systems in Florida and the manageability of the system over the long term will need to be investigated. These advanced production systems seem better adapted to production of fresh fruit and less appropriate for large-scale production of processing oranges. Many groves were planted at high density in Florida following the freezes in the 1980s. Early production was much higher than at conventional spacings, but when no freezes occurred in subsequent years, groves became unmanageable and growers reverted to more conventional tree spacings. Such advanced plantings would still have to be managed by removal of HLB-affected trees and application of insecticides for psyllid control. To be viable, these plantings would need to be located at some distance from sources of inoculum, (e.g. by conversion of pasture to citrus orchard).

Example of currently or recently supported research projects and relevant results: Plantings were made in 2006 at the Southwest Florida Research and Education Center comparing tree densities of 545, 198, and 151 per acre, with early and late oranges on different rootstocks. These plantings are being evaluated for yield, production costs and other parameters.

One promising high density planting system, the Open Hydroponics System (OHS), was developed in South Africa (Katz, 2008). For applications in Florida, OHS requires adaptation to Florida's summer rainy season and sandy soil. High density plantings using the OHS are being evaluated by Arapaho Farm Management, Inc. (http://www.arapahocitrus.com/ohs.html) on Florida's east coast.

A currently supported project (principal investigator Schuman) is concerned with intensively managed citrus production systems for early high yields and vegetative flush control in the presence of HLB.

Examples of possible future research projects: Flying Dragon trifoliate is the only commercially used rootstock that reduces tree size substantially and may be useful in these systems. Dwarfing of citrus trees by inoculation with viroids has been studied extensively in Australia and elsewhere (Hutton et al., 2000; Semancik, 2003; Hardy et al., 2007). Viroid species-rootstock combinations could be investigated as a means to reduce tree size to allow for closer tree spacing and earlier production and to adapt this approach for use in OHS.

Related information and references: Most of the current focus of alternative horticultural systems for citrus is on hydroponic-like systems for reducing the size of the tree root ball (Aki et al., 2008; Land and Water Australia, 2008) and on reducing tree size (Hutton and Cullis, 1981; Hutton et al., 2000; Semancik, 2003; Hardy et al., 2007)

Suggested support mechanism: Investigator-initiated proposals.

Time to outcome: Many years of investigation and grower experience would be needed before such systems could be recommended and widely utilized.

Other sections of this report containing related information: Chapter 2, p. 26–27 (landscape management), Chapter 3, p. 86 (cultural practices).

Recommendation NI-9. Support demonstration of RNA interference effects for possible suppression of ACP.

More than a decade ago, the phenomenon of RNAi was demonstrated in several phyla and now is recognized as a general and fundamental regulatory mechanism of biology. In the application of RNAi to negatively affect ACP feeding on citrus, the most feasible approach would involve transformation of citrus to accumulate, in phloem tissue, double-stranded RNA (dsRNA) corresponding in nucleotide sequence to an essential ACP gene. Under this scenario, the ACP RNAi system would use the dsRNA to interfere with the accumulation of ACP messenger RNA corresponding to the essential gene, resulting in mortality, morbidity or fecundity reduction of ACP. It is possible that protection could be achieved by transforming citrus rootstock to which untransformed scion would be grafted. Having available sequences of ACP genes (see *Recommendation NI-11*) will contribute to this approach.

Developing methods for effectively delivering RNAi-active molecules to ACP presents the greatest challenge to the application of RNAi technology. Experimentally, dsRNA administration to insects for initiation of RNAi is usually by injection (Begum et al., 2009) or by topical application (Pridgeon et al., 2008), but practical application would be by ingestion of dsRNA from phloem. RNAi activity from ingested dsRNA has been demonstrated in several systems (Price and Gatehouse, 2008; Zhou et al., 2008). Both predominantly single-stranded and highly structured phloem-located RNA molecules, their long distance transport in the plant, and in some instances their functions, have been demonstrated (Kalantidis et al., 2008; Banerjee et al., 2009; Turgeon and Wolf, 2009), so potential sources of RNA signals for transgene-mediated dsRNA delivery to phloem tissue are known, as are phloem-specific promoters (Chakraborti et al., 2009; Srivastava et al., 2009). Primary cell cultures from ACP have been reported (Marutani-Hert et al., 2009a). ACP cells in culture could provide a test bed for selection of sequences for their effectiveness against ACP.

The goal of Recommendation NI-9 is to establish proof-of-concept for this potentially new approach to ACP management. Fortunately, proof-of-concept can be established without use of transgenic citrus by taking advantage of transient expression and detached leaf uptake approaches.

Discussion of the Merits of NI-9

Favoring rationale: As is indicated above, citrus cultivars showing resistance to *C*Las and ACP are likely to be a critical part of effective management for HLB in the long term. Currently, there is no obvious path to creating ACP-resistant citrus by conventional breeding, and the RNAi approach could provide one form of ACP-resistant citrus.

Disfavoring rationale: Insect species vary in their susceptibility to the RNAi approach, and the susceptibility of ACP is unknown. Where an effect of dsRNA on the insect has been demonstrated, the concentrations of dsRNA that have been supplied have typically been close to 1 microgram per milliliter (1µg/mL). Presumably only much lower concentrations of dsRNA could be achieved in phloem fluid of transgenic citrus. There is no established method for transforming any plant to generate dsRNA in its phloem sieve tubes, so this technology would be needed before RNAi can confer ACP-specific insecticidal capability. Artificial diet-feeding of psyllids, which would be convenient for the testing of a variety of dsRNA constructions, is not readily accomplished for ACP.

Example of currently or recently supported research projects and relevant results: Three currently supported HLB research projects are aimed at identifying sensitive target sequences and administering dsRNA to a psyllid for the purpose of showing detrimental changes in phenotype. One of these projects is developing a method that will allow many different RNA sequences to be tested by a transient expression approach, which could uncover target sequences for which ACP would show a strong sensitivity, reducing the need for high level dsRNA accumulation in the transgenic plant.

Examples of possible future research projects: Investigations of systems for delivery of transgenic RNA (and proteins) to phloem sieve tubes would benefit RNAi work as well as other aspects of citrus improvement.

Related information and references: Demonstration of an RNAi action in insects was accomplished first by injection of dsRNA. However, Araujo et al. (2006) prepared dsRNA corresponding to a 548-bp segment of the *Rhodnius prolixus* (Chagas disease vector, Hemiptera) salivary nitrophorin-2 gene and were able to show a phenotype (reduction in blood coagulation time to about one-quarter of control values) both by injection and by feeding of the dsRNA, at a concentration of 1 µg/mL. RNAi action has been demonstrated in aphids (Mutti et al., 2006; Mutti et al., 2008) and whiteflies (Ghanim et al., 2007). RNAi has also been reported to be active against coleopteran and lepidopteran insects fed dsRNA in their diets (Baum et al., 2007; Mao et al., 2007).

Suggested support mechanism: The extensive basic research needed to provide proof-of-concept for plant-generated dsRNA with anti-psyllid RNAi capability will be best supported by competitively awarded grants.

Time to outcome: Demonstration of deleterious action of injected dsRNA against intact psyllids likely will be reported shortly. Similar action by dsRNA artificially introduced into plant phloem should follow within a year or two. Protection based on dsRNA synthesis and phloem secretion in a transgenic plant, if sufficient dsRNA can be generated, will likely require years of research.

Other sections of this report containing related information: Chapter 3, p. 83 (RNA interference); Appendix J (research on ACP management).

Recommendation NI-10. Develop *in vitro* culture techniques for *C*Las to facilitate experimental manipulation of the bacterium for insights into gene function.

The short-term culture of *C*Las *in vitro* has been reported, but culture of *C*Las over multiple transfers apparently has not been accomplished. Availability of *in vitro* culture technology for *C*Las as a general laboratory method could facilitate the finding of new insights into the physiology of this organism and possibly identify new points of vulnerability for control strategies.

Discussion of the Merits of NI-10

Favoring rationale: If the *in vitro* culturing of *C*Las were to become sufficiently facile, *C*Las could be considered to have been "isolated" and Koch's postulates could be completed, with *C*Las (presumably at that point becoming *Las*, no longer "*Candidatus*") identified unequivocally as the causal agent of HLB. Additionally, creating pure cultures of the bacterium *in vitro* should lead to improved understanding of this organism and to its genetic transformation, which would allow production of green fluorescent protein-expressing or otherwise marked strains and to the testing of individual genes for their role in citrus infection and virulence, ACP infection, and other functions of the bacterium.

Disfavoring rationale: This recommendation is not as strongly supported as it might have been just a few years ago because modern biology has provided enhanced capabilities for analysis of *C*Las characteristics in its natural environment, i.e., the infected plant or insect. Such analyses are likely to provide relevant information about *C*Las that would not readily be obtained from plate or liquid cultures.

Example of currently or recently supported research projects and relevant results: There are at least five currently funded projects aimed at culturing *C*Las, taking different approaches but usually attempting to mimic conditions of plant phloem or the insect body.

Examples of possible future research projects: It is possible that variations on the approaches that have given limited increase of *C*Las *in vitro* could provide a starting point to which many variations could be applied with the goal of supporting unlimited increase of *C*Las in culture. Availability of the *C*Las genome sequence may yield enough clues about the bacterium's metabolism to direct the incorporation of specific compounds into trial synthetic media.

Related information and references: Limited *in vitro* colony transfer of *C*Las has been reported using a solid medium containing citrus vein extract (Sechler et al., 2009).

Suggested support mechanism: Either a standard research grant approach or an incentive prize could be considered.

Time to outcome: Considerable funds and effort have been invested in attempts to culture *C*Las. The time necessary to achieve success is uncertain.

Other sections of this report containing related information: Chapter 3, p. 68 (*C*Las culture); Appendix J (research on *C*Las culture)

Recommendation NI-11. Sequence, assemble and annotate the ACP genome to provide a basis for new approaches to ACP management.

Recommendation NI-11 is intended to create information on which to base future strategies for ACP suppression or interference of *C*Las transmission by ACP (*Recommendation NI-7*).

Discussion of the Merits of NI-11

Favoring rationale: Where the complete genome of an animal is available, whether it be of humans or a small invertebrate (Opperman et al., 2008), benefit has been derived almost immediately from the investment in the form of genes as targets for disease amelioration or life cycle functional interference, in addition to new understanding of the organism as a whole. Thus far, a number of insect genome sequences are known, including the fruitfly, *Drosophila melanogaster;* honey bee, *Apis mellifera;* malarial mosquito, *Anopheles gambiae;* red flour beetle, *Tribolium castaneum;* and silkworm, *Bombyx mori* (Hart and Grosberg, 2009). The genome of the pea aphid, *Acyrthosiphon pisum* is also available (IAGC, 2010). For ACP, genome information could help identify targets likely to be accessible in gut cells and sensitive to interference by RNAi approaches (*Recommendation NI-9*), targets for chemical control and prophylactics or therapeutics, and factors that may be involved in nymph survival or ability to support *C*Las in ACP (*Recommendation NI-7*). The first determination of the haploid genome size for a phytophagous psyllid, *Pachypsylla venusta*, the hackberry petiole gall psyllid, was reported (Nakabachi et al., 2009) as 724 million base pairs, suggesting that sequencing the ACP genome could be readily accomplished using standard techniques.

Disfavoring rationale: Genome projects require significant initial investment and continuous supervision by expert management to coordinate selection of the source of genomic DNA, preparation of various types of genomic libraries, management of samples, the efforts of sequencing facilities, and quality controls. Significant bioinformatics capabilities for assembling and annotating the sequence are also required. As was noted, the complete genome sequence of an insect pest of plants has been accomplished only for the pea aphid, and as yet, there has been no demonstration of suppression of such insects based on genome information.

Example of currently or recently supported research projects and relevant results: A psyllid genomics consortium (http://www.ars.usda.gov/pandp/people/people.htm?personid=11768) has been started, and several datasets derived from ACP sequences are available at the National Center for Biotechnology Information (NCBI) (website www.ncbi.nlm.nih.gov).

Examples of possible future research projects: The value of a genome sequence lies in the ability of researchers to predict functions of genes from sequence alone and to identify genes that may be targets for manipulation of the organism—in the case of ACP, suppression. The genome sequence of a human parasitic nematode has been subject to bioinformatics analysis using the very extensively studied, free-living model nematode, *Caenorhabditis elegans*, as the reference for gene function information. The analysis revealed about 600 drug target candidates (Kumar et al., 2007). Similarly, the ACP genome sequence could be "mined" for insecticide targets, RNAi targets, and new types of targets that might be revealed in the analysis, using the very extensively studied model insect *D. melanogaster* (www.fruitfly.org) and the recently sequenced pea aphid genome (http://www.hgsc.bcm.tmc.edu/project-species-i-Pea%20Aphid.hgsc) as reference sequences.

Related information and references: An EST library of ACP has been prepared representing about one-fifth of the likely transcripts of the organism (Hunter et al., 2009), and sequences suggesting the presence of a virus of ACP were detected (Marutani-Hert et al., 2009b).

Suggested support mechanism: Presuming that there is more than one group seeking support for genome sequencing, funds should be awarded competitively.

Time to outcome: Significant sequence information, and perhaps even the entire genome sequence, could be accomplished in less than two years using current high-throughput methods.

Other sections of this report containing related information: Appendix K.

RECOMMENDATIONS FOR RESEARCH AND TECHNOLOGY WITH THE POTENTIAL TO ADVANCE HUANGLONGBING MITIGATION IN THE LONG-TERM

Given the urgency of the HLB situation in Florida, most investments in research and management are necessarily near-term. However, continuing investments in research aimed at longer term solutions remains important. In addition to the four long-term recommendations below, completing the citrus genome sequences and exploiting them to create usable breeding systems for commercial citrus (*Recommendation NI-4*) should be a priority. The genome sequence, when combined with high-throughput SNP genotyping and other additional information, can facilitate improvement of Florida citrus varieties.

TABLE 4-4 Long-Term Recommendations

L-1. Support development of transgenic HLB-resistant and ACP-resistant citrus.
L-2. Support development and testing of bactericides, therapeutics or SAR activators.
L-3. Support analysis of ACP behavior, ACP-plant interactions and ecology to enhance the knowledge base available for new ACP management strategies.
L-4. Explore possible control strategies based on release of modified psyllid males.

Recommendation L-1. Support development of transgenic HLB-resistant and ACP-resistant citrus, including creating suitable anti-*C*Las and anti-ACP genes.

It is generally agreed that *C*Las- or ACP-resistant citrus would provide the ideal long- term management tool for HLB. Currently, the most promising path to developing resistant citrus involves genetic engineering (GE). Advances in GE approaches include techniques to utilize mature tissue, to bypass the juvenile stage and accelerate the transformation and regeneration process (Cervera et al., 1998). Other research groups are exploring transgenes that may confer resistance to pathogens other than *C*Las or greater tolerance to low temperatures. To expedite rapid screening of candidate trait genes, *Citrus tristeza virus* (CTV)-derived vectors are available for stable transient expression in citrus (Folimonova et al., 2007) and can be used to provide a testbed for gene constructions designed to have anti-*C*Las or anti-ACP activity.

Although there are non-citrus plants that demonstrate resistance to *C*Las or ACP, such plants are not likely to be sources of genes useful in citrus because of inter-species incompatibility and difficulties in isolating such genes. Sequences encoding anti-*C*Las transgenes are more likely to be anti-microbial peptides and proteins (AMPs) of animal, plant, microbial or bacteriophage origin (Canny and Levy, 2008; Conesa et al., 2009; Soehnlein, 2009; Mao et al, 2010). Similarly, there are many possible sources for sequences that could encode anti-ACP proteins (Sauvion et al., 2004; Staniscuaski et al., 2005; Down et al., 2006; Gonzalez-Zamora et al., 2007; Follmer, 2008), including venom components, lectins, bacterial spore proteins, protease inhibitors, and vitamin-binding proteins.

From a practical standpoint, GE strategies should consider traits that can be delivered via rootstocks that can be deployed across a number of scions. Several rootstocks are in use in Florida, and each rootstock would need to be transformed independently unless conventional genetic crossing is available. Nevertheless, incorporation of the transgene into a few rootstock lines would be considerably less expensive than transforming many scion lines. Of course, replacement of existing trees is an additional significant expense and an expense that will likely continue well into the future. In-arch grafting would allow rootstock substitution for existing trees, reducing the need for replacement. An orchard citrus tree may have a one-hundred year life span, demanding durable resistance. Thus, strategies for constructing transgenic, disease-resistant citrus should involve the pyramiding of transgenes with different mechanisms of action in order to minimize the chances of *C*Las or ACP overcoming resistance.

As is described in Chapter 3, CTV vectors have shown exceptional longevity for expression of foreign genes in citrus, so CTV vectors may provide benefits beyond serving as a testbed for candidate transgenes. An effective CTV construction conferring anti-*C*Las or anti-ACP biology or transmission ability could be introduced into existing orchard trees by conventional grafting techniques, providing protection against HLB and even therapeutic effects without tree replacement. Thus, CTV-vector-mediated HLB mitigation strategies warrant serious attention. In addition, the research strategies adopted, whether using conventional transformation or CTV, should be designed with full consideration of achieving the necessary downstream regulatory approvals and intellectual property freedom-to-operate.

Discussion of the Merits of L-1

<u>Favoring rationale:</u> Genetically engineered resistance, whether by conventional transformation or introduction by CTV vector, has the greatest potential of available technologies for long-term HLB disease management (Singh and Rajam, 2009). For conventional transformation, creating high-throughput transformation protocols with an accelerated regeneration timeframe of transgenic citrus is a critical first step. Recent advances in citrus transformation technology and the availability of potential resistance-conferring transgenes will allow more rapid progress than would have been possible even five years ago.

<u>Disfavoring rationale</u>: A realistic evaluation of GE strategies suggests that although there is promising research in this area, there are significant technical challenges that will need to be overcome. The need, particularly in the long run, for durable resistance requires that multiple transgenes be developed to provide pyramided protection against both *C*Las and ACP. Creating multiple resistance transgenes will be technologically very demanding.

Any new transgenic crop is subject to extensive regulatory procedures before commercialization can be approved, requiring significant investments of money and time. Even if a transgenic citrus line is approved for commercialization, grower and public acceptance remains uncertain.

<u>Example of currently or recently supported research projects and relevant results</u>: In 2008–09, about $2.1 million dollars were invested in citrus research focused on transgenic and viral/bacterial vector mediation of citrus resistance to HLB. See projects listed in Appendix J, transgenic and viral/bacterial vector mediation of citrus resistance to HLB.

Examples of possible future research projects: Future research projects stemming from this recommendation include an assessment of the feasibility of using transgenic rootstock to deliver disease resistance to a wide variety of scions. Research strategies will require testing promoters and signal sequences that mobilize the transgenic molecules across the graft union. Both proteins and molecular signals of other types have been demonstrated to traverse a graft union (Golecki et al., 1998; Prassinos et al., 2009), supporting the concept of rootstock-delivered anti-*C*Las or anti-ACP molecules.

There is no regulatory precedent for a system in which resistance is conferred on the scion from a transgenic rootstock. As is likely for any other new regulatory situation, rootstock protection may have special requirements for field trials and data collection, which will need funding.

Since intellectual property is another key element in developing transgenic citrus, it may be important to fund future research projects that validate technologies with freedom-to-operate that may be used as foundation technologies in citrus bioengineering. Here, we anticipate it may be necessary to support research projects that analyze the international legal issues surrounding the key transformation technologies and other projects that test the functionality of these technologies in citrus; for example, plant selection genes, *Agrobacterium*-mediated transformation, plant transformation vectors and transcription promoters.

Another future research project should consider the development and/or deployment of marker-free transformation technologies. There is debate within the international community about whether marker-free plants will offer consumer acceptance advantages or facilitate regulatory registration, but there is emerging regulatory resistance to allowing antibiotic resistance genes in Europe.

Suggested support mechanism: It is clear that research in genetic engineering of citrus has a long horizon and will require a multi-disciplinary team approach with funding to laboratories working on the evaluation of resistance gene strategies, developing efficient transformation protocols, field-testing transgenic trees, developing regulatory information and ensuring intellectual property freedom-to-operate. To ensure coordination of these activities, there should be a funding program with a guidance board dedicated to identifying and funding a consortium of laboratories to quickly advance the development and deployment of transgenic citrus trees.

Time to outcome: GE strategies are recognized as one of the areas with greatest potential and should be supported immediately. This long-term objective probably will require 10–15 years of research investment and regulatory approval and public education efforts, although if current field trials were successful and accelerated approval processes were taken up, commercialization of *C*Las- or ACP-resistant citrus could occur in less time. Implementing a collective research effort that also focuses on intellectual property and regulatory compliance will accelerate development, field testing, and registration.

Other sections of this report containing related information: Chapter 3, pp. 78–83 (transgenic citrus, anti-*C*Las genes), and pp. 84 (CTV vectors).

Recommendation L-2. Support development and testing of bactericides and other therapeutics or activators of systemic acquired resistance for control of *C*Las.

Development of a bactericide or some other curative product that could be applied for control of HLB would provide many advantages. Tree removal would not be necessary and control of psyllids might not need to be so rigorous. However, there are no systemic bactericides, except for antibiotics, that have been registered for use on citrus or any other crop. The bactericides that currently exist are copper products that have been used for centuries and antibiotics that were developed in the 1940s. Manufacturers of agrichemicals have invested only to a limited extent in the development of such products and there are no potentially useful products of this type on the horizon. Activators of systemic acquired resistance (SAR), such as salicylic acid and phosphorous acid, are available on the market and some are registered for use on citrus. These products have generally provided only a low level of control of bacterial diseases. The systemic insecticide, imidacloprid, currently used for psyllid control on citrus, has shown some SAR activity for control of citrus canker and may be of some value for HLB mitigation.

Discussion of the Merits of L-2

Favoring rationale: Bactericides could provide a solution like no other: curative action effective on the already *C*Las-infected tree, eliminating the need for other control measures for HLB. If such products could be applied by foliar sprays, conventional techniques familiar to growers could be used for treatments.

Disfavoring rationale: No currently available product is likely to provide the potency, at an affordable price, needed to make other control measures unnecessary. Even if symptoms were reduced to an acceptable level, *C*Las would probably persist in the trees and continue to be sources of inoculum for infection of other trees. Also, the bacterium might be able to develop resistance to any product applied, so this control measure might not endure. Tree injection of antibiotics was used for a time for control of HLB in South Africa, but control was not complete, application was difficult, and the practice was eventually discontinued. SAR products would be prophylactic and are unlikely to provide the level of control necessary to avoid other control measures. A practical product would need to be persistent in effect and be of relatively low cost.

Example of currently or recently supported research projects and relevant results: Current HLB projects include four on SAR (Graham, Lu, Rouse, and Stansly) and one on screening compounds for anti-*C*Las activity (Powell).

Examples of possible future research projects: High-throughput model systems (see *Recommendation NI-5*) have the potential to sort through many hundreds of compounds to identify those with prophylactic or therapeutic capabilities but with minimal adverse consequences to the plant.

Related information and references: SAR is a widely recognized phenomenon but one that has found only limited application in plant protection (Durrant and Dong, 2004; Francis et al., 2009). Similarly, there have been few applications of therapeutics in the control of citrus diseases (Schwarz and von Vuuren, 1971; Buitendag and von Broembsen, 1993; Layden, 2009) or any plant disease.

Suggested support mechanism: Investigator-initiated proposals to a competitive grants agency.

Time to outcome: Some products available now could prove helpful short-term, but development of highly effective products would likely require many years of research.

Other sections of this report containing related information: Chapter 3, pp. 83, 86 (systemic acquired resistance); Chapter 3, p.87 (model systems); Appendix J, (citrus response to infection); Appendix K (systemic acquired resistance genes in transgenic grapefruit).

Recommendation L-3. Support analysis of ACP behavior, ACP-plant interactions and ecology to enhance the knowledge base available for new ACP management strategies.

Control of ACP by currently available conventional insecticides will not likely be sufficient for long term mitigation of HLB. Newer insecticides will be helpful, but their use will be constrained by costs of the insecticides and their applications, uneven distribution of the insecticide within trees, the evolution of insecticide resistance and damage to non-target organisms and the environment. To overcome those barriers, a better understanding of the *C*Las/ACP/citrus-HLB system is needed. This recommendation is aimed at extending and fortifying the most promising observations obtained under Recommendation NI-1c, and at bringing them to practical application through the development of a mathematical model of the plant-vector-pathogen complex.

Mathematical models that incorporate data such as those expected to be generated under Recommendation NI-1c (behavior of ACP adults and nymphs; interactions of ACP, *C*Las, citrus trees, other organisms and the environment) provide a tool through which the results of behavioral ecology can be applied to disease mitigation (Gonzalez-Zamora et al., 2007; Follmer, 2008; Mitchell et al., 2009; Cunniffe and Gilligan, 2009). Although information on ACP behavioral ecology that falls short of what may be needed for a model will still be instructive for ACP management in the near-term, the construction of a mathematical model for the *C*Las/ACP/citrus-HLB system should be a long-term goal of HLB research. It is recognized that the complexity of pathogen transmission by an insect vector will make the task very challenging.

Discussion of the Merits of L-3

Favoring rationale: Compared with other phytophagous hemipterans, relatively little is known about the behavior and ecology of psyllids, including ACP, so what new information may be collected will expand the knowledge base significantly with likely application to HLB mitigation.

Disfavoring rationale: Translating results from research on ACP behavioral ecology into ACP management and HLB mitigation, including the construction of an appropriate mathematical model, will be difficult and likely time consuming.

Example of currently or recently supported research projects and relevant results: No studies targeted on the behavioral ecology of the immature stages of ACP are funded. The only references regarding nymphal behavior are anecdotes published in extension publications (Grafton-Cardwell et al., 2006; Rogers and Stansly, 2006).

Related information and references: Several references examine behavioral aspects of psyllids and other small piercing and sucking herbivorous insects (Dawson et al., 1990; Novak, 1994; Cocroft, 2001; Luft et al., 2001; Grafton-Cardwell et al., 2006; Rogers and Stansly, 2006; Guedot et al., 2008; Wenninger et al., 2008; Wenninger et al., 2009a; Wenninger et al., 2009b).

Suggested support mechanism: Competitive grants seem most appropriate. Several investigators are currently funded to study various aspects of the biology, ecology and behavior of adult ACP. It is suggested that these researchers meet, discuss the merits of the above recommendations, and expand their studies with existing funding to include the behavioral ecology of immature ACP.

Time to outcome: New information about the behavior of ACP immature and mature forms can be obtained and exploited within a year or two. For example, newly discovered attractants, of psyllid or plant origin, could be included in psyllid traps to improve trapping efficiency.

Conversely, discovered repellents could be used alone or in combination with insecticides to better manage psyllids.

Other sections of this report containing related information: Chapter 3, p. 76–78 (ACP behavioral ecology).

Recommendation L-4. Explore possible psyllid control strategies based on release of modified psyllid males.

The sterile insect technique (SIT) (the release of x-ray-irradiated males), has proven to be successful in several management strategies for agriculturally important pests. Newer techniques, which exploit genetically transformed insects, produce populations of males that are not debilitated by irradiation and therefore can compete with wild males. Recommendation L-4 explores the potential for the newer technologies to suppress ACP using these techniques.

Discussion of the Merits of L-4

Favoring rationale: The psyllid is a high-priority target for HLB management because it is an essential link in the infection cycle and is potentially vulnerable to a variety of control measures. In SIT, the target insect is raised on a mass scale and males are segregated, irradiated to induce sterility, and released into the area of the target insect population to compete with wild males for the available females. Separating the males is not trivial for many insect species. A recently developed alternative technology employs insects genetically transformed for a female lethal gene. This gene is suppressed by a component of the insect diet during rearing, that component being withheld at the last generation before release to yield a male-only population not capable of fathering females (Fu et al., 2007).

Disfavoring rationale: Compared to nymphs, adults acquire the HLB bacterium at a low efficiency, but not zero, so large releases of psyllids could increase HLB transmission marginally. Psyllids can reproduce to very high populations on some trees. Psyllid females mate serially. Therefore, the psyllid population would need to be reduced significantly before release of modified males, and, even so, large releases would likely be required to be effective in reducing the population in the next generation. Raising psyllids on a large scale at low cost will almost certainly require an artificial diet. No one has succeeded in rearing psyllids from egg to adult on a synthetic diet, much less the industrial-scale rearing that would be required to raise psyllid males for release. To date, SIT has not been developed for a phytophagous hemipteran.

Example of possible future research projects: (a) develop an artificial diet for rearing ACP from egg to adult and then develop procedures for producing psyllids on a large scale; (b) isolate female-specific gene or genes from ACP and prepare constructs for conditional expression of a lethal protein under control of the corresponding female-specific promoter; (c) rear the transgenic ACP on the artificial diet under conditions (e.g., diet component) that suppresses expression of the female-lethal gene; (d) prepare males for release by altering conditions to promote expression of the female-lethal gene; and (e) on a large-scale, release ACP males transgenic for a female-lethal trait into orchards with a reduced ACP population.

Related information and references: An alternative reproductive sterility system was developed for the medfly (*Ceratitis capitata)* based on transgenic embryonic lethality. About 60 transgenic constructions were tested, of which several lines developed larval and pupal lethality. A line that showed complete embryonic lethality nevertheless was highly competitive against wildtype medfly in cage tests (Schetelig et al., 2009).

Suggested support mechanism: This area is NOT recommended for support because there is no indication that psyllids can be reared on a synthetic diet, let alone on a large scale. However, development of an artificial diet for use in large-scale rearing of ACP could justify support. Even if large-scale rearing were to be achieved, the future possible research projects indicated above present tremendous technological challenges.

Time to outcome: Long-term.

Other sections of this report containing related information: Chapter 3, p. 71 (sterile insect technique).

5

Strategies for Implementing Plans for Mitigation of Huanglongbing and Other Problems in Citrus Production

IMPLEMENTING PLANS FOR HUANGLONGBING MITIGATION

Implementation in Other Industries

Inevitably, the organization and structure of the Florida citrus industry, as well as federal and state agencies, have molded the structure, shape and direction of research and development (R&D) intended to benefit the industry. As the citrus industry has developed, it has become increasingly dependent on R&D. The structure of the citrus industry contrasts with the structure of a typical R&D-dependent industry, which is made up of individual companies, each with its own captive R&D capability, both in-house and outsourced. Company R&D management is responsible and accountable for producing or procuring next-generational product advancements and improvements that will provide a competitive advantage relative to other companies in the same sector. R&D management also is charged with solving the technological problems that invariably arise in the course of the company's business. It is management's ultimate responsibility to prioritize, allocate, and, when conditions change, redistribute R&D resources in a manner that best serves the company's interests. R&D decision- making is facilitated by overall milestones and development phases incorporated into an overarching strategic plan which is intended to ensure that new products and services enter the market with critical delivery timing. The strategic plan and progress against milestones are reviewed on a regular basis across all stakeholders to ensure goals will be met. The Florida citrus industry has several characteristics that distinguish it from the model described above. The Florida citrus industry is necessarily fragmented because it consists of dedicated fruit producers who operate independently. The producers do not have a captive R&D function, per se. Rather, they rely on a system of research grants from a number of different sources, including their box tax contributions, to sponsor and drive research. Consequently, the sources of research funding are also fragmented. This structure, a natural consequence of widely dispersed ownership of the means of production, presents challenges to achieving focus, balance and integration in R&D topics, goals and lines of responsibility and authority. Decision-making tends to be slow and bureaucratic and not well-suited for crisis management.

The health care industry also has a dispersed structure. Individual physicians represent a highly fragmented "industry". In times of a health care crisis a central authority, often the Centers for Disease Control, takes charge and provides national, or even international, leadership. The authority takes on a highly focused, high urgency tactical and strategic role and helps to coordinate initiatives already underway at state and local levels. Since huanglongbing (HLB) is an international problem, and without question of great relevance to the Americas and US, a strike-force like structure may be helpful to coordinate activities, maximize resource impact, and to set research direction, priorities, and review progress toward providing a sustained solution to HLB and other technical challenges posed to the citrus industry. Experience in health care may provide guidance to the citrus industry under the concept of **best practices**. The intent of a best practices approach is to observe efforts within and between industries in order to identify those practices that produce improved results. Clinical researchers closely track results of trials of a rigorously defined new treatment protocol, trials that often include direct comparison against the current treatment of choice. Results are offered to the medical community for critical consideration. When the data convincingly demonstrate the superiority of a new protocol, the new protocol is expected to replace the old and become the treatment of choice. In the case of HLB, the identification of best practices offers relatively near-term benefits. Practices elsewhere that are documented to produce a better result in some aspect of crop management can be adapted and applied to Florida crops fairly quickly. An important byproduct of best practices documentation is its ability to influence and shape public policy relevant to the citrus industry. For instance, politically contentious environment management techniques (e.g., mandated destruction of diseased trees and abandoned orchards) used elsewhere with success can lend graphic support for similar practices in Florida.

Achieving Agreement in Support of Implementing Plans

It is generally agreed that a concerted effort must be made to reduce the impact of HLB in the near- and intermediate-term, and there is general acceptance of three parts to current HLB mitigation efforts (Brlansky et al., 2009), as discussed in previous chapters: use only nursery stock that is free of *Candidatus* Liberibacter asiaticus *(C*Las), detect and remove infected trees, and manage the Asian citrus psyllid (ACP) vector.

Pathogen-Free Nursery Trees

The Citrus Health Response Program developed protocols for production of disease-free trees. As of January, 2008, all citrus nursery trees in Florida are required to be produced in screened enclosures from pathogen-free budwood and rootstock seed. These procedures have been highly effective in eliminating the spread of HLB by commercial nursery stock. Movement of the disease with ornamental plants such as *Murraya* has also been greatly reduced. The *C*Las-free tree program must be counted as an implementation success.

Detection and Removal of Infected Trees

Identification and removal of diseased trees is absolutely essential in any program of HLB mitigation, but, especially in the northern and central areas where incidence until very recently has been low, the practice has not been fully implemented, as it should be. The need to remove infected trees creates a hardship, especially for small growers. Some growers are now abandoning tree removal programs since they have proven costly and the incidence of HLB has continued to rise. However, at Southern Gardens, the first and originally the most seriously affected plantation, that practice continues to be effective. In their experience, even with a rigorous tree removal and psyllid control program, HLB incidence initially rises due to the long latent period of the disease. Only after 2–2½ years of such practices is the incidence of HLB substantially reduced (M. Irey, United States Sugar Corp., Clewiston, FL, personal communication). Current attempts to maintain production by applications of nutritional and anti-bacterial materials are completely understandable from the point of view of the individual grower but are counter-productive to the interests of the industry as a whole. In addition, efforts should be made to encourage the removal of abandoned groves wherever possible. Once incidence in a grove reaches 5–10 percent, removal of trees is unlikely to deter the rate of spread of the disease sufficiently to be sustainable. Homeowners should be encouraged to eliminate citrus as an ornamental and replace it with other fruit trees and ornamental shrubs. To reduce inoculum to a level that will allow a return on investment from replant trees will require implementation of tree removal on an area-wide basis (Recommendation O-1).

Control of the Psyllid Vector (Recommendation NI-1)

There are many issues related to ACP suppression. For insect-transmitted diseases such as HLB, ACP must be suppressed to low levels to reduce spread of the disease. Sufficiently low levels are unlikely to be achieved in continental areas with biological control alone. Nevertheless, maintenance and protection of biological control species, including those remaining in abandoned groves, is necessary to reduce populations of ACP and other insect pests year round. Whenever possible, products which have the least effect on beneficials should be selected and application timing should take their life cycles into account. More economical and environmentally friendly low-volume and aerial applications of insecticides and the rotations of insecticides with different chemistries, to reduce development of insect resistance, have been and are being developed. Fortunately, a large number of soil-applied and foliar insecticides are available for ACP control and inclusion in recommended insecticide protocols (Rogers et al., 2010). What practices should be followed? Given the severity of the HLB problem, some growers will continue to treat in a prophylactic manner and when populations are high based on trap counts. However, recent work has shown that well-timed sprays during the dormant period can be very effective at reducing the critical spring ACP population, and dormant period applications may make it possible to reduce the overall number of annual sprays. Well-timed sprays during flushes of growth also can be very effective in reducing ACP populations. Implementation of ACP suppression programs will be best accomplished using an area-wide management approach (next section).

Area-wide Management Programs (Recommendation O-1)

Area-wide management programs are important, even essential, for effective mitigation of HLB in Florida. Experience in both Brazil and Florida indicates that it is difficult to control HLB if neighboring groves have high levels of the disease (Belasque Jr., 2008). Thus, it will be important for neighboring growers to coordinate their efforts for both ACP suppression and tree removal. Implementation of area-wide management programs will require local support and support from the entire industry as well. Only by implementation of an area-wide approach is it likely that best practices for both infected tree removal and ACP suppression will be disseminated and enforced.

ACCELERATING PRODUCT DEVELOPMENT AND COMMERCIALIZATION: GRANTS VS. CONTRACT PRIZES AND ALTERNATIVES

Alternative Models for Support of HLB Research

The Florida citrus industry has correctly identified research as the vehicle to deal with HLB. An open question remains as to whether the type of research being supported is in balance or will be adequate to the challenge. When the knowledge needed to solve a particular problem does not exist within an organization[1], various methods may be used to build that knowledge. For an individual company, existing internal R&D capability may be expanded to include initiatives that may be expected to plug knowledge gaps. This approach is often applied when the nature of the knowledge to be gained is central to the organization's strategic interests, the discoveries are progressive, the new knowledge gained can serve as a building block on which additional progress through can be built, internally developed results are key to gaining competitive advantage and outright ownership of intellectual property (IP) and maintaining proprietary technical expertise. In other circumstances, and when the industry consists of many independent operators, outsourcing research may be more effective. Research may be outsourced under any of four types of agreements (Table 5-1). Implementing any of these may have great potential for stimulating research results that significantly advance the mitigation of HLB.

Research Grants

Research grants, most often made to universities, or government or private research institutes provide a way to support formation of a solution. This is especially true if the solution is in the category of a breakthrough or a long-standing, widely recognized unmet need. A key characteristic of research grants is that the deliverables are only directionally specified by the sponsor and a distinct, actionable solution to the articulated problem may or may not result. What is expected of the grant recipient is a certain level of effort dedicated to the topic that results in original research. The research leads to relevant publications, possibly patentable intellectual property, papers, reports and presentations. Research grants contribute to the preexisting knowledge base and may reach a tipping point that actually solves the underlying problem. This research is most often performed at arms length from any R&D laboratory that the sponsor may have. Depending on provisions negotiated between the sponsor and grant recipient, commercialization rights and preferential access to IP may be stipulated. Because sponsored research grants most often result in new knowledge rather than a breakthrough innovation that

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[1] *Organizational* and *internal* for purposes of this section are meant to apply also to the decentralized R&D initiatives taking place in academic settings on behalf of citrus growers and governmental agencies.

TABLE 5-1 External Research Vehicle Characteristics

	Grant	Sponsored Research	Contract Research	Inducement Prize
Nature of task	Fill knowledge gap or identify an entirely new research direction; experiment driven	Fill knowledge gap; experiment driven	Solve specific problem; harvest knowledge	Solve specific problem; output driven
Number of potential solvers	Few	Few	Few	Possibly many
Risk bearer	Sponsor	Sponsor	Sponsor	Solver
Sponsor control	Low	Low to moderate	High	Low
Output	New knowledge; occasional breakthrough	New knowledge; occasional breakthrough	Specified deliverable	Specified deliverable if winner emerges
Intellectual property ownership	University/researcher	Negotiable among university/researcher/sponsor	Sponsor	Sponsor
Confidentiality	Low	Low	High	Moderate to-high
Urgency/speed	Low	Low to moderate	High	High; deadline driven
Work plan	Directional, discovery driven, iterative	Directional, discovery driven, iterative	Roadmap, schedule driven	Opportunistic, hit or miss

has direct commercial application, such grants by industry sponsors are often considered a form of philanthropy that serves society, enhances company image, and may serve the company's R&D self-interest if something having commercial utility happens to result. From the perspective of industry, research grants are viewed as supporting pre-competitive basic and to a lesser degree, applied research. It is for this reason that most research grants at US universities are not sponsored by industry, but rather by the federal government through various agencies such as the National Science Foundation (NSF), National Institutes of Health, Department of Defense, Department of Energy, and the US Department of Agriculture (USDA). On occasion, foundations, trade organizations, and affiliation groups may sponsor research grants. The Florida citrus industry is an excellent example of this latter circumstance. Individual growers contribute to research grants via an allocation process, in this instance tied to a "box tax" (RGG, 2009; USDA-CSREES, 2009).

Sponsored Research

Sponsored research, compared to grants, places a greater emphasis on applied research. It is a nuanced form of a research grant. The sponsor selects this structure in instances where the external academic laboratory has highly specialized expertise, often in the form of sophisticated equipment, analytical techniques, or cutting edge know-how that complement those of its own laboratories. In this variant of a research grant the sponsor and the university laboratory anticipate a higher degree of interaction between the academic laboratory and the sponsor's technical staff, including possible intellectual property commingling. Relative to a grant, sponsored research makes greater use of schedules, milestones, and details about ownership of outputs. The sponsor is eager to avoid the circumstance in which its independently produced know-how or even trade secrets flow to a competitor. The sponsored research agreement places great emphasis on confidentiality and review of manuscripts prior to journal submission. The sponsored research mode often is selected when the sponsor has a sense of urgency about a project.

Contract Research

Contract research, as its name implies, is performed under a legally binding agreement between the parties. It states the sponsor will pay the outside researcher a predetermined fee for specific services. Unlike the sponsored research contracts discussed above, contract research is considerably more prescriptive. It can involve university researchers or private research organizations. Intellectual property that may arise is usually owned by the sponsor or assigned exclusively to the sponsor. Publication rights may be at the discretion of sponsor. Some would argue that contract research is really more in the realm of development than research per se because the recipient may believe that it has within its control the know-how to produce the deliverables stipulated in the contract. In any event, it is an effective tool by which to expand currently existing capabilities of the sponsor because it permits the sponsor to tap into expertise it does not and cannot easily possess. By arranging multiple contracts on the same topic, the sponsor may create parallel paths toward a solution of a difficult problem to improve the chances of success.

Inducement Prize

Some "problems" have proven to be particularly vexing for an industry or society. The solution is elusive for those closest to it, both internal and external to the sponsor. In such instances, the use of inducement prizes may prove effective. Described simply, inducement prizes are created by an announcement to the public in the form of a detailed description of the problem to be solved, a specification of what constitutes a solution and usually a deadline for submission of proposed solutions. A team of judges selects which solution, if any, merits awarding of the prize. In some instances awards can be offered for the first practicable solution submitted and to the best solution provided prior to the deadline (Hotz, 2009; McKinsey and Company, 2009).

The use of inducement prizes is not new. In 1714, the British Parliament created The Longitude Prize for a solution that could provide longitude information on board a ship out of sight of land to within ½ degree. A prize of £20,000, a substantial amount in that day, was offered. Eventually in 1773, William Harrison was determined to be the winner of the prize, although his chronometer-based innovation was shown to meet the award specification much earlier. A food preservation prize was created by Napoleonic France that resulted in the canning process that is still in use. More recently, The Ortig Prize of $25,000 was offered for the first non-stop flight between New York and Paris. Charles Lindbergh won the Ortig Prize for his flight on May 21–22, 1927.

Inducement prizes are in resurgence. An estimated combined purse in 2007 of $165 million was available in the science, engineering, climate, and environment sectors out of a total of $315 million (also including aviation, space, arts, and others). The success rate for inducement prizes is relatively high. According to Karim Lakhani from the Harvard Business School, out of 166 problems posted by a firm specializing in the creation of inducement prizes for clients, nearly 30 percent of the problems were successfully solved. Although sometimes criticized for being overly commercial and even frivolous, inducement prizes have gained respectability. In 1999, a National Academy of Engineering workshop endorsed the concept (NAE, 1999). In 2007, the National Research Council issued a report, "Innovation Inducement Prizes at the National Science Foundation," that recommended the use of such prizes to strengthen the innovation engine in the US and that the NSF adopt this approach among its more traditional funding methods (NRC, 2007).

Currently offered inducement prizes carry substantial awards. They are often over $100,000 and range into the millions of dollars. Since the prize is only awarded for success, the financial risks to the sponsor are relatively low, amounting to administrative and promotion costs if no solution emerges. In fact, risk is transferred to those choosing to participate. Another advantage of such prizes is they draw interest from individuals and teams outside the usual community of scientists associated with the problem. Talent that is geographically dispersed and may not be known to the sponsor can also participate. Such prizes bring fresh eyes to the problem, and they can leverage technologies already developed in other industries. Also, outsiders are not unduly biased by "conventional wisdom" that may serve to limit avenues of exploration for a solution. The deadline of an inducement prize imposes a desired sense of urgency. In these ways inducement prizes cast a wide net for practicable solutions to vexing problems.

A number of unresolved aspects relating to HLB have the potential for accelerated resolution via the inducement prize route. Approaches that would significantly delay the spread of HLB or extend the economic viability of commercial orchards without maintaining sources of *C*Las are highly desirable. Writing the specifications for an inducement prize that would encourage discovery of such approaches would be challenging.

As described elsewhere in this report, a mobile, non-contact sensor system that would identify infected trees as the sensor was driven past trees could aid in early removal of infected trees and slowing spread of the disease. Another example is an award for culturing *C*Las for a specified number of generations and providing the growth media and cultured pathogen available to the scientific public. A prize could be created for developing and introducing into citrus a gene for resistance to *C*Las or ACP, with specifications of success in fewer than the 10 years now anticipated for this accomplishment and for minimal alterations to the varietal type.

Each type of research support structure offers different levels of accountability, speed, risk, and desired outcome. Current initiatives are heavily weighted in the grant category, which arguably may be overweighed. Figure 5-1 presents a decision tree to aid in the selection of the type of external research approach that best suits a given type of research or development objective.

INTELLECTUAL PROPERTY MANAGEMENT WITH THE AIM OF STIMULATING DEPLOYMENT OF HUANGLONGBING TECHNOLOGY

Intellectual property (IP) is a widely used term. The term also has precise meanings rooted in US and international law. *Intellectual* refers to a product of the human mind. *Property* connotes something that the "owner" can sell, license, rent, hold exclusively for him/herself, trade, or even give away. Governments have long recognized the importance of IP as a vehicle to encourage innovation and to contribute to society's advancement. In an effort to encourage individuals to take technical, scientific, agricultural, and other forms of creative risk, governments offer limited monopolies for useful discoveries and creative works. Patents are the oldest form of such intellectual property rights. Although the exact origin of a patenting system is unclear, it is generally believed a rudimentary system began in Renaissance Italy to protect innovations in glass blowing developed by Venetian artists. This system spread to other parts of Europe. The first recorded patent was awarded in 1449 to John of Utynam by the English government for a novel method to manufacture stained glass (Thompson Reuters).

The framers of the US Constitution recognized the importance of intellectual property for a developing nation. Article 1, Section 8 of the Constitution states:

> *"The Congress shall have power… to promote progress of science and useful arts by securing for limited times to authors and inventors the exclusive right to their respective writing and discoveries."*

Patents are the most recognized type of IP. IP can also take the form of trademarks which protect product "brands"; copyrights which protect written works, artwork, music, presentations, and similar forms of unique expression; or trade secrets, usually reserved for commercially relevant recipes, formulas, or processes (www.wipo.int/about-ip/en). The criteria for obtaining a patent are highly technical and are beyond the scope of this report. It is, however, relevant to present briefly several key aspects of patents that may not be widely understood and have relevance in the context of HLB. The granting of a patent by the United States Patent and Trademark Office (USPTO) is an exclusionary right. That is, the owner of a valid patent secures for the statutory lifetime of the patent the right to keep others from making, selling, or distributing the specifically defined inventive subject matter, known as the "claims", of the granted patent. It does not confer on the owner the right to actually make, sell, or distribute, except in certain circumstances. An example is needed here. Let's say the inventor develops the idea of and method for manufacturing a white sidewall tire for cars. She submits to the USPTO the properly drafted and executed application, usually prepared by a patent attorney. After a determination by a patent examiner at the USPTO (a process that is very exacting, thorough, and may take a few years) that the application represents a useful and novel innovation, the inventor is granted a US patent for a white sidewall tire as articulated in the claims. Can the inventor now sell white sidewall tires? The answer depends on the circumstances. If someone already holds a valid patent on a tire for which the whitewall version is an improvement, the answer is "no", unless she secures permission (usually in the form of a license) from the owner of the "prior art" tire patent her invention builds upon. The answer is "yes" if there are no prior art patents that her invention builds on still in force (that is, relevant prior art tire inventions have expired or never existed), or if hers is a fundamentally new breakthrough idea.

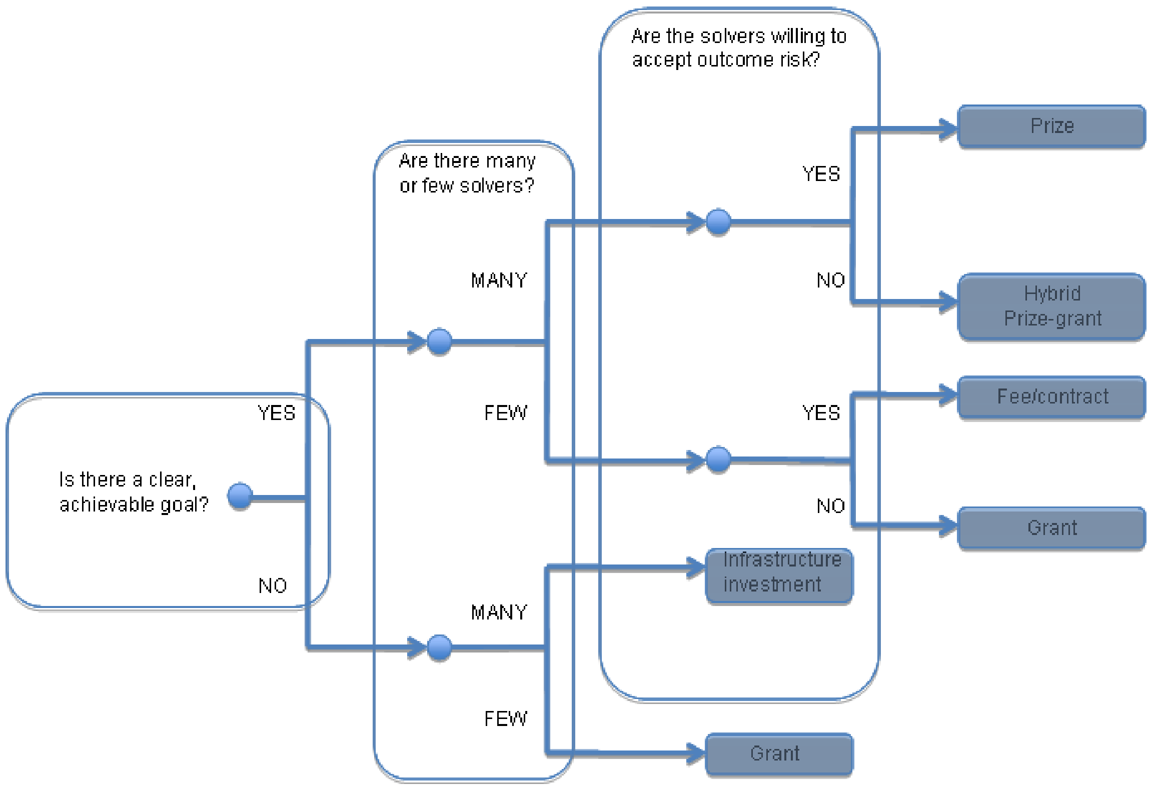

FIGURE 5-1 Decision tree for using an inducement prize rather than other types of research or development support.
Exhibit taken from McKinsey and Company, 2009.

The example above brings out the notion that many patents represent improvements to already existing core ideas. The ability to create and gain patent protection for an improvement on a patented item is a fundamental benefit underlying the concept of a government-issued patent. In return for a limited monopoly on the inventive subject matter, the inventor, as part of the application process is required to teach the world how to make and practice his/her invention. In fact the inventor is obligated to enumerate the best teaching known to him/her. This requirement enables another person to understand the technology underlying the granted patent and, if capable and so motivated, to invent an improvement, thereby spurring technological progress for the benefit of society.

Patents are an important element of the commercialization process, but within a context. The patent requires the innovative "flash of light", but many more steps are required to "reduce to practice" and actually commercialize the concept into a product that relies on the patented invention. Matters such as robust design, manufacturability, scale-up, cost to produce, reliability, safety, effectiveness, and securing of regulatory clearances and registrations must be resolved as part of the commercialization process. All of these factors represent financial and opportunity risk for the manufacturer. Absent patent protection, owned or licensed, which keeps fast followers from simply copying successful innovations, companies would have few incentives to invest in the risky, costly and time consuming commercialization process.

The HLB Research and Development Enterprise and Intellectual Property

Effective management of HLB will require a multi-pronged approach. Measures such as scouting for early signs of infection, prompt removal of diseased trees, the use of disease-free nursery stock, and other crop management practices generally are in the public domain, as are public policies and regulations regarding disposition of abandoned groves and ornamental plantings. Other aspects, such as genetically modified trees, new insecticides targeted at controlling the psyllid vector, new antibiotic preparations, or novel detection technologies require careful consideration of IP, particularly patents. These later aspects require a costly development cycle to bring them to market. In some instances navigation of and investment in complex regulatory processes to prove safety and effectiveness are also mandatory.

Research aimed at growing an understanding of HLB and for ways to mitigate or even cure the disease is highly distributed in the US and around the world. Sponsors of important research are also large in number and only loosely affiliated and coordinated, if at all. While most research initiatives are unlikely to have direct commercial applicability (note: this in no way impugns the scholarly importance of the work) research which does result in patentable discoveries need to be handled correctly if patents are to be sought. It must be emphasized again that without protective patents in place, companies will be very reluctant to invest in developing new technologies, particularly if there are large pre-investments required. Granting organizations need to articulate in their award documents expectations and responsibilities regarding inventions, if they do not already do so. It is also important to impress upon researchers, their staff, and host institutions the fundamentals of securing patents. Researchers must know what steps they need to follow in order to comport with the rigorous application requirements set forth by the USPTO and patent bodies elsewhere.

A number of actions by the inventor(s) may serve to bar patent eligibility or invalidate their already issued patents. Among the most common of these are: premature public disclosure of the invention and listing the wrong inventors in the patent application. The US patent law requires that the inventor must file a patent application no later than one year after a public disclosure of the invention. European law and most of the rest of the world requires an application be filed in their jurisdiction before a public disclosure of the invention[2]. Premature public disclosure of the invention often comes in the form of a presentation or literature publication. Submitting a presentation abstract, assuming it discloses the invention, to symposium organizers or an article for review by journal editors can serve as a statutory bar to eventual patenting or be the event that invalidates a patent already issued if filing steps and timetables were not satisfied. Disclosures via guest lectures, letters, informal conversations with individuals not directly involved with the invention (e.g., support staff, technicians), or offers to sell the invention may also be sufficient for a public disclosure to have occurred (MIT-TLO, 2006). Failure to accurately identify the list of inventors may invalidate a patent. Criteria and legal standards for inventorship are set by the USPTO and similar offices in other geographies. Determination of the legal inventors is a task best left to a patent attorney following interviews with individuals associated with the invention.

Not all potentially patentable ideas should be submitted for patenting. The subject matter

[2] Some technicalities apply regarding a "priority patent application" but are beyond the scope of this discussion. Suffice it to say an oral or written public disclosure may serve as an absolute bar to receiving a patent or invalidating one previously issued.

may be too narrow, too esoteric, or perhaps too impractical. What's more, securing a patent is very expensive. Technology-based patents can cost upwards of $20,000 to secure. Many countries impose periodic maintenance fees for already issued patents. While the fee for any one invention may be relatively small, organizations holding many patents must consider whether the fees in aggregate are affordable. It is not uncommon to allow a patent lapse by not paying the maintenance fee due to well thought-out business reasons. Reasons include lack of present and strategic relevance, inability to secure licensees, lack of competitive advantage offered by the patent, and so forth.

Deciding what to patent is difficult. Large companies usually employ internal patent review boards to prioritize inventions. Patent board members include at least one patent attorney and typically representatives from engineering, marketing, and corporate development. Patent boards must possess both tactical and strategic expertise concerning not only technology but also the potential market for goods or services exploiting the patent. HLB research has both industry and government sponsors and is carried out primarily at academic institutions with disparate IP policies, making a centralized patent board impractical. Nevertheless, it is important that each funding source is aware of the role that patents play in the search for solutions to the HLB scourge and for each funding source to articulate patent strategies for their grant awardees that will preserve commercialization incentives.

Regulatory Approval

With transgenic citrus development as one of the research areas currently being funded by the citrus industry, it is imperative that IP and regulatory approval issues are considered by the industry early on. The previous section underscored the importance of seeking patents to protect IP. However, also of great importance is a Freedom to Operate (FTO) audit which will determine if patent infringement has occurred during the development of transgenic citrus. As pointed out by Bennett (2009), citing "Golden Rice" as an example of a transgenic crop that was found to have infringed on 70 proprietary technologies during its development (Kryder et al., 2000), patent infringement has serious consequences on the commercialization of the transgenic crop. Aside from this, navigating the complex process of deregulation also merits considerable attention. To date, USDA Animal and Plant Health Inspection Service (APHIS) has deregulated (i.e. made accessible to growers and consumers without the need for a permit) only two transgenic tree species, papaya (*Carica papaya*) and plum (*Prunus domestica*). The development of transgenic papaya (SunUp and Rainbow), with resistance to the papaya ringspot virus (PRSV), began in the mid 1980s; the deregulation process began in 1995 and was completed in 1997 (Gonsalves, 2004). The development of transgenic plum (Honeysweet), with resistance to Plum Pox disease, a viral disease transmitted by aphids and by grafting, spanned 15 years; it was deregulated by APHIS in 2007 (Scorza et al., 2007).

Three federal agencies are involved in the deregulation of a transgenic crop, USDA-APHIS, which has authority over the cultivation of transgenic crops, the Food and Drug Administration, which has authority over transgenic crops that are used as food, and the Environmental Protection Agency which has regulatory authority over transgenic crops that provide protection against pests and diseases. The deregulation process is complex and, as shown the by the examples above, may take as little as two years to as much as 15 years to be completed. According to Bennett (2009), the lack of "*a dedicated staff and expertise*" available to the researcher seeking to deregulate his or her transgenic crop contributes to the inefficiency of the process.

Patent infringement and other intellectual property issues that can impede the development and commercialization of transgenic crops can now be more capably addressed by the public sector with assistance from institutions such as the Public Intellectual Property Resource of Agriculture. With regards to achieving a deregulated status, there is one program that was specifically established to provide help with navigating the regulatory process for transgenic specialty crops. In their website, the Specialty Crops Regulatory Assistance is defined as “a collaborative, public-private effort to assist public and private-sector developers of biotechnology-derived specialty crops in their efforts to complete the complex US regulatory process for commercialization of biotechnology-derived crops” (http://www.specialtycropassistance.org).

Below are strategies that Bennett (2009) has recommended, some of which may have already been taken up by the citrus industry:

- Adopting a translational research philosophy
- Identifying partnerships that can integrate complementary technologies
- Establishing an IP policy for HLB research
- Establishing relationships with regulatory agencies

Preparing for HLB Management Based on Genetically Modified Citrus

As has been noted previously at several points in this report, the development of *C*Las-and ACP-resistant citrus varieties, or citrus rootstock that confers resistance on the scion, presents the greatest hope for a viable long-term solution to the HLB problem. At this time, there is no obvious path to achieving this goal by conventional citrus breeding. Therefore, approaches are favored that are aimed at developing transgenes capable of conferring resistance and introducing them into appropriate citrus lines. There are technical challenges associated with identifying effective transgenes and introducing them into citrus, particularly introducing them into mature citrus so that stock for budding or planting can be made available in a reasonable period of time. It is likely that these challenges can be met in the next few years. The difficulties in obtaining on IP and FTO are presented in the previous section, as are approaches to overcoming these difficulties. Should all of the technical and IP requirements be satisfied, there remains the potential barrier of unfavorable public acceptance.

In 2008, the thirteenth year of commercialized transgenic crop production in the US, transgenic plantings had expanded to 154 million acres (62.5 Mha) and include field crops (soybean, corn, cotton, canola, alfalfa, and sugarbeet), a vegetable crop (squash), and an orchard crop (papaya) (http://www.isaaa.org/resources/publications/briefs/39/executivesummary/default.html). More than 90 percent of the 2008 US soybean crop was accounted for by transgenic, herbicide-tolerant varieties. Given the ubiquity of soybean derivatives in processed foods, there probably are very few residents of the US who have not consumed transgenic soybean. More than 60 percent of the cotton and corn crops were transgenic, providing additional transgenic dietary contributions. These facts do not mean that transgenic foods are fully accepted by consumers in this country. The organic food industry’s niche exists in large measure because of the claim that organic food is “GMO-free.” Several well-known activist organizations have taken a stance against genetically modified foods in general and would likely attempt to raise opposition in the general public to juice from transgenic oranges.

Citrus appropriately has a reputation for providing health-benefitting products. This reputation, as helpful as it is in marketing citrus, may not be helpful in achieving public acceptance of products derived from genetically engineered citrus. What may be helpful is that the consumers of Florida orange juice almost all reside in the US, possibly the industrialized country that has been the most accepting of transgenic crop products. Although transgenic versions of minor crop species have been created no genetically engineered minor crop plant, other than squash and papaya, has been deregulated for commercial production. Possibly the only approach that could smooth the path for public acceptance of transgenic citrus products would be a fact-based, carefully vetted public education campaign that reveals the benefits of transgenic crop production. These benefits include a substantial reduction in pesticide use and production costs associated with these crops and, in some instances, even the ability to grow the crop in a particular area. Clearly, this education effort should not be borne by the citrus industry alone but could be an appropriate program of a consortium of minor crop producers who may anticipate benefits from transgenic versions.

CONSEQUENCES AND BACKUP PLANS SHOULD HUANGLONGBING COUNTERMEASURES PROVE TO BE INSUFFICIENTLY EFFECTIVE

The Florida citrus industry is an important component of the state's economy. Its economic impact is estimated at $9.3 billion. The industry provides approximately 80,000 full-time equivalent jobs (grove employees, seasonal pickers, haulers, processors, and packers). These workers earn a combined annual wage of $2.7 billion. This translates into approximately 1.5 percent of the state's wage income (Norberg, 2008). While some may view the affected stakeholders fairly narrowly (i.e., growers, processors, and affected workers), in the event the Florida citrus industry goes into a sustained decline, there is, in reality, a much broader affected constituency. Beyond dollar terms, citrus products are a healthful and tasty component of our diet, and citrus represents a cherished way of life for many growers and a key part of the state's wholesomeness image for consumers, tourists, and prospective residents. Part of Florida's "brand" and reputation is tied to citrus, which is a distinguishing feature for the state. While brands can be repositioned, it takes a long time and must be done carefully. Thoughtful scenario and contingency planning among stakeholders, including the aforementioned groups, developers, and those at the urban/rural interface, will serve to make painful transitions more orderly in the event that sufficient HLB countermeasure do not materialize.

A substantial reduction in Florida citrus production has national economic implications. Eighty-five percent of the world's orange juice production occurs in São Paulo State, Brazil, and Florida. Reduced Florida production presumably would result in increased imports, especially from Brazil and China. Similar considerations apply to Florida's fresh citrus crops. As described in Chapter 2, citrus production in Florida has been under pressure from freezes, storms, diseases, decreasing water supplies, increasing costs of production, and urbanization. The number of juice processors has also declined.

Experts suggest HLB is the most formidable challenge confronting commercial citrus in Florida and in many other citrus regions in the world as well. Unlike the instantaneously devastating, but somewhat localized, effects of hurricanes and freezes, unchecked HLB is expected to have a progressive impact on the commercial viability of Florida citrus. Some have suggested that HLB could be the tipping point that irreparably cripples the Florida industry if HLB countermeasures prove to be insufficiently effective, are too expensive to employ, or do not come into practice soon enough. The purpose of the narrative that follows is to explore possible back-up plans and strategies should the Florida citrus industry we know today prove to be unsustainable under pressure from HLB.

Tree loss and production yield decline are expected to be gradual, the actual rates being dependent on the aggressiveness with which current HLB management methods are applied. A key presumption is that, in the absence of newly developed and more effective management tools, Florida citrus will not disappear but will shrink to a fraction of its present size. Growers and orange juice producers will need to develop alternative business models that reflect this decline and still keep a critical mass of production facilities open. We are not able to recommend a specific grower/producer model for the future but only to identify some elements that may be a part of that future.

The decline in the number of orange juice producers in Florida is expected to continue as fruit tonnage declines are exacerbated by HLB. Various HLB mitigation measure will be adopted and should contribute to reducing tree loss. However, groves will probably become concentrated in a few areas in which inoculum sources can be minimized and one or more processing plants can be sufficiently supplied. Forward- looking land management strategies will be crucial as growers seek to extend productivity of existing groves and perhaps plant new groves in areas less challenged by nearby sources of inoculum. Presumably, high density plantings with their necessary modified irrigation systems (Chapter 3) will be a part of the picture. The short time to production for high density plantings could keep the grove ahead of HLB and could save some processing plants from shutting down.

Growers will need to partner with local and state lawmakers to devise plans, appropriate incentives and, quite possibly, ordinances that deal with issues such as abandoned groves and to minimize new abandonments without dealing appropriately with existing trees when individual growers elect to exit commercial production. In addition, land use plans and strategies that permit an orderly transition to economically feasible alternative uses should be considered as part of a long range master development plan that comports to each community's vision of its future.

Marketing campaigns for Florida orange juice will need to confront the greater importation of non-domestic product. This will require a delicate and planned balance of messages. On the one hand, the virtues of drinking orange juice irrespective of source must be conveyed to the public. On the other hand, the advantages of 100 percent Florida-sourced juice will need to be articulated clearly. At some point there is likely to be a pronounced price differential between imported and domestic juice due to the expected higher cost of US production as additional HLB management methods come into play. How will this be explained and domestic product positioned to US consumers? The issue of US and non-US juice blends will also need to be attended to in a way that does not confuse or alienate consumers as they make supermarket choices of which juice or drink they purchase.

RECOMMENDED MEASURES FOR THE CONTROL OF NON-HUANGLONGBING DISEASES AND PEST PROBLEMS

HLB is by far the most serious disease affecting the Florida citrus industry and should be given priority. Nevertheless, the other diseases and pests that currently occur in the state, or that occur elsewhere in the world and threaten the industry, should not be ignored.

Citrus canker and HLB are both diseases that have been introduced in recent years and are currently affecting the industry, but other diseases occur elsewhere that could be potentially damaging to Florida citrus. Diseases such as citrus variegated chlorosis (Hartung et al., 1994), leprosis (Bastianel et al., 2006), Pseudocercospora fruit and leaf spot (Seif and Hillocks, 1998), and black spot (Peres and Timmer, 2003) could be serious problems for the industry if introduced (Appendix E).

Most diseases and pests of citrus have been introduced into new areas by unauthorized, purposeful importation of budwood and other propagating materials or accidental movement on plant material brought in by tourists and other visitors. Except in specific cases, seed does not represent an important means of movement of citrus diseases. Likewise, commercially packed fruit is not a major means for movement of diseases, but can be for Mediterranean fruit fly, whose larvae actually live within the fruit (Thomas et al., 2007). Since vectors of leprosis, tristeza virus, and citrus variegated chlorosis are already present in Florida, there is less concern about the introduction of such insect pests. The efforts of regulatory agencies to intercept and prevent movement of pathogens should be increased and supported by the industry.

The primary means to eliminate spread of systemic diseases is by the use of disease-free budwood and good nursery practices. The current system of production of propagating material has been effective in reducing the dissemination of many plant pathogens and pests. This program should be supported and expanded to prevent movement of any newly introduced pathogens or pests.

Citrus canker has become widespread in the state and is an important problem for the industry especially for fresh fruit production. The primary controls for this disease are the use of windbreaks and application of copper products (Dewdney and Graham, 2009; Rogers et al., 2010). Windbreaks are highly effective for canker control but have not been widely planted in the state. The planting of windbreaks for citrus canker control, especially in fresh fruit growing areas should be encouraged.

Many important diseases caused by fungi, such as postbloom fruit drop and Alternaria brown spot already occur in the state, but others such as Pseudocercospora fruit and leaf spot, black spot, and sweet orange scab that occur elsewhere still threaten the industry. Most of the diseases caused by fungi, whether they already occur in the state or could be introduced are controlled by application of fungicides or, in few cases, by utilization of resistant cultivars (Timmer and Brown, 2000). More effective fungicides for control of these diseases and other control measures need to be developed. Predictive models, such as PFD-FAD for postbloom fruit drop (Peres et al., 2005) and the Alter-Rater for Alternaria brown spot (Timmer et al., 2000), help reduce fungicide applications and improve disease control. Development of such systems for other diseases would be beneficial. Currently, there are few commercial cultivars available for control of fungal diseases. Efforts to develop such cultivars should be expanded and supported.

METRICS FOR PROGRESS

Expand the Intellectual Base Addressing HLB

A critical need is to expeditiously expand the research base that is looking for solutions to HLB. Increasing intellectual capacity that mitigates HLB requires striking international and multidisciplinary scientific collaborations. Competitive research funding will attract new researchers and new ideas, potentially from divergent fields, that have the potential to make rapid progress. Rigorous evaluations, from scientific and industry perspectives, of research priorities, proposals, and progress are critical to ensure timely advancement in the field. An important metric will be the number and caliber of scientists entering the HLB field and progress of scientific advancements against HLB disease.

Encourage Global Cooperation

Mitigating HLB will require global cooperation in intellectual development, research funds, and biological resources. Particularly in the area of genetics and breeding, the capacity to evaluate and maintain large and diverse germplasm blocks is critical. This can be facilitated by access to counter-seasonal climate and to shared fields. A collaborative network of citrus geneticists and breeders coordinated from a single site can provide more strategic use of resources, reduce redundancy and accelerate progress in genetic improvement. The development of global collaborations and the leveraging of external research resources towards common objectives should be a measure of progress.

Translational Research

A research program that is targeted towards practical outcomes should embrace a philosophy of translational research from its inception. In concrete terms, translational research programs are characterized by investment in fundamental research that is required to understand the problem at hand but with close linkage to applied research programs that can rapidly translate the results to the field. This requires interdisciplinary research teams and the development of a research community that communicates frequently and easily. A culture for information sharing, publication, and scientific workshops and meeting should accelerate research and development. In addition, if the program is sponsoring transgenic research it should explicitly integrate an assessment of the "downstream" IP and regulatory issues at the outset of each project and invest in the expertise and strategies that will facilitate the deployment of successful research targets. A measure of progress should include early progress in adopting a translational research philosophy and in establishing processes of information sharing and accountability in terms of timelines and progress towards research deliverables.

Glossary

Acquisition For a circulative pathogen like "*Ca.* Liberibacter" spp., passage of ingested pathogens through the gut epithelial cells into the vector haemocoele.

Agrobacterium A bacterium that normally causes crown gall disease in a variety of plants.

Agrobacterium-mediated transformation A method of genetic engineering that involves the use of a plasmid (called *Ti* plasmid) from Agrobacterium. This plasmid has been made incapable of causing disease but can carry foreign DNA into susceptible plant cells.

Anti-bacterial peptide A compound composed of two or more amino acids that can destroy bacteria or suppress its growth.

Apomictic Not requiring the union of male and female gametes (i.e. fertilization) during reproduction.

BAC library (bacterial artificial chromosome) A cloning vector (i.e. small piece of DNA into which a foreign gene can be inserted) that is propagated in a bacterial host and is used in large scale genome sequencing projects**.**

Best management practices Methods or techniques that have been shown to be the most practical and the most efficacious.

Biomarker A substance that is used as an indicator of disease or a biological state.

Box Tax Tax paid by the grower for each box of harvested citrus, used to fund the Florida Department of Citrus activities.

Budwood A stem used as source of bud for grafting.

***Citrus tristeza virus* (CTV)** A single stranded RNA virus from the group Closterovirus, which is known to cause economically important plant diseases. The causal organism of the citrus tristeza disease.

Genetic engineering A technology that is employed to alter the genetic material of living cells for the purpose of making them produce new substances or perform new functions.

Genome annotation The process of determining the locations of genes and coding regions in a genome and their functions**.**

Genome sequencing The process of determining the DNA sequence of an organism's genome (i.e. total hereditary material).

Graft To unite a shoot or bud (i.e. scion) to an established plant (i.e. stock) by insertion or by attachment.

Graft inoculation Introduction of a pathogen to a healthy plant by inserting a bud which is infected with the pathogen (i.e. grafting).

Gram-negative bacteria Bacteria that turn red or pink when subjected to Gram staining protocol, a reaction due to the structural composition of their cell walls.

Haemocele The blood-filled body cavity of arthropods.

Inoculation…For **"***Ca.* Liberibacter" spp. is the passage of pathogens in saliva from the salivary glands into phloem sieve elements via salivation.

Heterozygosity The condition wherein dissimilar alleles (forms of a gene) are present at one or more loci on homologous chromosomes.

Latent period (plant) Time in the plant from pathogen inoculation by vector, graft or mechanical transmission to the time of pathogen expression by symptoms or detection by diagnosis (e.g. serology, PCR, TEM).

Homoptera An insect sub-order comprised of plant-feeding insects with sucking mouthparts**.** Its members include aphids, leafhoppers, cicadas, and scale insects.

Phytoplasma Formerly known as 'Mycoplasma-like organisms' or MLOs, are bacteria that lack cell wall which thrive in plant phloem tissues and some insects.

Polymerase chain reaction (PCR) A technique used to increase the amount of a specific DNA sequence or DNA region by separating the DNA strand into two and incubating it with oligonucleotide primers and DNA polymerase**.**

Pure culture In vitro growth of only one type of microorganism.

Pyrosequencing A DNA sequencing technique that depends on the detection of pyrophosphate release during DNA synthesis.

Retention For **"***Ca.* Liberibacter" spp. is the act of pathogen moving through haemocoele and infecting various organs including the salivary glands.

RNAi (RNA interference) A defense mechanism in plants, fungi, and animals against foreign double-stranded RNA, such as viruses. The process is characterized by the prevention of messenger RNA (mRNA) translation by specialized protein complexes in the host.

Rootstock A root or part of a root that is used as the base in plant propagation (i.e. grafting).

Scion A twig or shoot with buds that is attached to the rootstock during grafting.

Systemic acquired resistance (SAR) Resistance to disease exhibited by the whole plant after inoculation with a pathogen that causes a localized response or after the application of certain chemical agents.

Transformation Change in the genetic structure of an organism by the incorporation of DNA from another organism, not necessarily of the same species.

Transmission For *Ca.* Liberibacter spp. is the acquisition, retention (over a latent period), and inoculation of the pathogen by the vector.

Transgenic An organism formed by the insertion of a gene obtained from another organism using recombinant DNA techniques.

Vector An agent, such as plasmid or virus, used to carry DNA into a cell; an insect that transmits a pathogen.

Vertical transmission Transfer of an infectious agent from parent to offspring via transovarial transmission and eliminates need for traditional acquisition by feeding on a pathogen-infected host.

References

Aki, T., M. Shigyo, R. Nakano, T. Yoneyama, and S. Yanagisawa. 2008. Nano scale proteomics revealed the presence of regulatory proteins including three FT-like proteins in phloem and xylem saps from rice. *Plant and Cell Physiology* 49(5):767–790.

Akimitsu, K., T.L. Peever, and L.W. Timmer. 2003. Molecular, ecological and evolutionary approaches to understanding Alternaria diseases of citrus. *Molecular Plant Pathology* 4(6):435–446.

Albrecht, U., and K.D. Bowman. 2008. Gene expression in *Citrus sinensis* (L.) Osbeck following infection with the bacterial pathogen *Candidatus* Liberibacter asiaticus causing huanglongbing in Florida. *Plant Science* 175(3):291–306.

Aleixos, N., J. Blasco, F. Navarrón, and E. Moltó. 2002. Multispectral inspection of citrus in real-time using machine vision and digital signal processors. *Computers and Electronics in Agriculture* 33(2):121–137.

Ali, S., and B. Mirza. 2006. Micropropagation of rough lemon (*Citrus jambhiri* Lush.): Effect of explant type and hormone concentration. *Acta Botanica Croatica* 65(2):137–146.

Aluja, M., A. Jiménez, M. Camino, J. Piñero, L. Aldana, V. Castrejón, and M.E. Valdés. 1997. Habitat manipulation to reduce papaya fruit fly (Diptera: Tephritidae) damage: Orchard design, use of trap crops and border trapping. *Journal of Economic Entomology* 90(6):1567–1576.

Alvarez, A.E., E. Garzo, M. Verbeek, B. Vosman, M. Dicke, and W.F. Tjallingii. 2007. Infection of potato plants with potato leafroll virus changes attraction and feeding behaviour of *Myzus persicae*. *Entomologia Experimentalis et Applicata* 125(2):135–144.

Al-Zadjali, T.S., R. Ibrahim, and A.K. Al-Rawahi. 2008. First record of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) from the Sultanate of Oman. *Insecta Mundi* 2008(0039):1–3.

AOML (Atlantic Oceanographic and Meteorological Laboratory). 2009. All U.S. Hurricanes (1851-2008). Miami: NOAA/AOML. Available online at http://www.aoml.noaa.gov/hrd/hurdat/ushurrlist18512008.txt. Accessed August 22, 2009.

Araujo, R.N., A. Santos, F.S. Pinto, N.F. Gontijo, M.J. Lehane, and M.H. Pereira. 2006. RNA interference of the salivary gland nitrophorin 2 in the Triatomine bug *Rhodnius prolixus* (Hemiptera: Reduviidae) by dsRNA ingestion or injection. *Insect Biochemistry and Molecular Biology* 36(9):683–693.

Asana, R.D. 1958. The citrus dieback problem in relation to cultivation of citrus fruits in India. *Indian Journal of Horticulture* 15:283–286.

Ashihara, W. 2004. Survival of Asian citrus psyllid, *Diaphorina citri* Kuwayama adults under winter temperature conditions in Central and South Kyushu. *Japanese Journal of Applied Entomology and Zoology* 48(2004):207–211.

Aubert, B. 1987. *Trioza erytreae* del Guercio and *Diaphorina citri* Kuwayama (Homoptera: Psylloidea), the two vectors of citrus greening disease: Biological aspects and possible control strategies. *Fruits* 42:149–162.

———. 1990. Integrated activities for the control of huanglungbin-greening and its vector *Diaphorina citri* Kuwayama in Asia. In Proceedings of the Asia Pacific international conference on citriculture, eds. B. Aubert, S. Tontyaporn and D. Buangsuwon, pp. 133–144, February 4–10, at Chiang Mai, Thailand. Rome: FAO-UNDP.

———. 1992. Citrus greening disease, a serious limiting factor for citriculture in Asia and Africa. In Proceedings of the 7th International Citrus Congress, pp. 817–820, n.d., at Acireale, Italy. Gainesville: University of Florida.

Aubert, B., and J.M. Bové. 1980. Effect of penicillin or tetracycline injections of citrus trees affected by greening disease under field conditions in Reunion Island. In Proceedings of the 8th Conference of the International Organization of Citrus Virologists, eds. E.C. Calavan, S.M. Garnsey and L.W. Timmer, pp. 103–108, May 13–31, at Australia. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsVIII/8th103_108.pdf. Accessed July 12, 2009.

Aubert, B., and X.Y. Hua. 1990. Rehabilitation of citrus industry in the Asia Pacific Region: Monitoring flight activity of *Diaphorina citri* on citrus and *Murraya* canopies. In Proceedings of the 4th Asia Pacific International Conference on Citriculture, eds. A. Bernard, T. Sanchai and B. D., pp. 181–187, February 4–10, at Chiang Mai, Thailand. FAO-UNDP, Rome.

Aubert, B., and S. Quilici. 1984. Biological control of the African and Asian citrus psyllids (Homoptera: Psylloidea), through eulophid and encyrtid parasites (Hymenoptera: Chalcidoidea) in Reunion Island. In Proceedings of the 9th Conference of International Organization of Citrus Virologists, eds. S.M. Garnsey, L.W. Timmer and J.A. Dodds, pp. 100–108, May 9–13, 1983, at Argentina. Riverside, CA: IOCV. Available online at http://www.ivia.es/ iocv/archivos/proceedingsIX/9th100_108.pdf. Accessed July 12, 2009

Aubert, B., and S. Quilici. 1988. Monitoring adult psylla on yellow traps in Reunion Island. In Proceedings of the 10th Conference of International Organization of Citrus Virologists, eds. L.W. Timmer, S.M. Garnsey and L. Navarro, pp. 249–254, November 17–21, 1986, at Valencia, Spain. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsX/10th249_254.pdf. Accessed October 27, 2009.

Aubert, B., A. Sabine, P. Geslin, and L. Picardi. 1984. Epidemiology of the greening disease in Reunion Island before and after the biological control of the African and Asian citrus psyllas. In Proceedings of the 5th Meeting of the International Society of Citriculture, Vol. 2, pp. 440–442, n.d., at São Paulo, Brazil. Gainesville: University of Florida.

Aubert, B., M. Grisoni, M. Villemin, and G. Rossolin. 1996. A case study of huanglongbing (greening) control in Réunion. In Proceedings of the 13th Conference of the International

Organization of Citrus Virologists, eds. P. Moreno, J.V. da Graça and L.W. Timmer, pp. 276–278, November 16–23, at Fouzhou, China. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsXIII/13th276_278.pdf. Accessed July 12, 2009.

Augier, L., G. Gastaminza, M. Lizondo, M. Argañaraz, and E. Willink. 2006. Presencia de *Diaphorina citri* (Hemiptera: Psyllidae) en el Noroeste Argentino (NOA) [Record of *Diaphorina citri* (Hemiptera: Psyllidae) in North West Argentina]. *Revista de la Sociedad Entomológica Argentina* 65(3-4):67–68.

Azevedo, F., F. Mourão Filho, B. Mendes, W. Almeida, E. Schinor, R. Pio, J. Barbosa, S. Guidetti-Gonzalez, H. Carrer, and E. Lam. 2006. Genetic transformation of Rangpur lime (*Citrus limonia* Osbeck) with the *bO* (bacterio-opsin) genen and its initial evaluation for *Phytophthora nicotianae* resistance. *Plant Molecular Biology Reporter* 24(2):185–196.

Banerjee, A.K., T. Lin, and D.J. Hannapel. 2009. Untranslated regions of a mobile transcript mediate RNA metabolism. *Plant Physiology* 151(4):1831–1843.

Barnett, C. 2007. Mirage: Florida and the Vanishing Water of the Eastern U.S. Ann Arbor: University of Michigan Press.

Bassanezi, R.B., A. Bergamin Filho, L. Amorim, N. Gimenes-Fernandes, T.R. Gottwald, and J.M. Bové. 2003. Spatial and temporal analyses of citrus sudden death as a tool to generate hypotheses concerning its etiology. *Phytopathology* 93(4):502–512

Bastianel, M., J. Freitas-Astua, E.W. Kitajima, and M.A. Machado. 2006. The *citrus leprosis* pathosystem. *Summa Phytopathologica* 32(3):211–220.

Baum, J.A., T. Bogaert, W. Clinton, G.R. Heck, P. Feldmann, O. Ilagan, S. Johnson, G. Plaetinck, T. Munyikwa, M. Pleau, T. Vaughn, and J. Roberts. 2007. Control of coleopteran insect pests through RNA interference. *Nature Biotechnology* 25(11):1322–1326.

Bedford, I.D., A. Kelly, G.K. Banks, D. Fuog, and P.G. Markham. 1998. The effect of Pymetrozine, a feeding inhibitor of Homoptera, in preventing transmission of cauliflower mosaic caulimovirus by the aphid species *Myzus persicae* (Sulzer). *Annals of Applied Biology* 132(3):453–462

Begum, K., B. Li, R.W. Beeman, and Y. Park. 2009. Functions of ion transport peptide and ion transport peptide-like in the red flour beetle *Tribolium castaneum*. *Insect Biochemistry and Molecular Biology* 39(10):717–725.

Belasque, Jr., R.B. Bassanezi, P.T. Yamamoto, S.A. Lopes, A.J. Ayres, J.C. Barbosa, A. Tachibana, A.R. Violante, A. Tank, Jr., C.L. Giorgetti, F. Di Giorgi, F.E.A Tersi, G.M. Menezes, J. Dragone, L.F. Catapani, R.H. Jank, Jr., and J.M. Bove. 2008. Factors associated with control of huanglongbing in São Paulo, Brazil: A case of study. International Research Conference on Huanglongbing12:1, 336–341.

Belasque, Jr., J., N. G. Fernandes, and C. A. Massari. 2009. The success of eradication campaign of citrus canker in São Paulo State, Brazil. *Summa Phytopathologica* 35(2):91–92.

Bennett, A.B. 2009. Intellectual property and regulatory approval of transgenic crops: New challenges for the public research sector in fulfilling its historical role in translational agricultural research. June 16, 2009. Unpublished manuscript. University of California, Davis.

Bextine, B.R., D. Harshman, M.C. Johnson, and T.A. Miller. 2004. Impact of Pymetrozine on glassy-winged sharpshooter feeding behavior and rate of *Xylella fastidiosa* transmission. *Journal of Insect Science* 4:1–6.

Bianchi, F.J.J.A., C.J.H. Booij, and T. Tscharntke. 2006. Sustainable pest regulation in agricultural landscapes: A review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society B: Biological Sciences* 273(1595):1715–1727.

Blasco, J., N. Aleixos, J. Gómez, and E. Moltó. 2007. Citrus sorting by identification of the most common defects using multispectral computer vision. *Journal of Food Engineering* 83(3):384–393.

Blua, M. J., and D. J. W. Morgan. 2003. Dispersion of *Homalodisca coagulata* (Hemiptera: Cicadellidae), a vector of *Xylella fastidiosa*, into vineyards in Southern California. *Journal of Economic Entomology* 96: 1369–1374.

Boina, D.R., E.O. Onagbola, M. Salyani, and L.L. Stelinski. 2009. Antifeedant and sublethal effects of imidacloprid on Asian citrus psyllid, *Diaphorina citri*. *Pest Management Science* 65(8):870–877.

Boller, T., and G. Felix. 2009. A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology* 60:379–406.

Boman, B.J., and L.R. Parsons. 1999. Microsprinkler experiences in Florida citrus. *Applied Engineering in Agriculture* 15(5):465–475.

Bonani, J.P., A. Fereres, E. Garzo, M.P. Miranda, B. Appezzato-Da-Gloria, and J.R.S. Lopes. 2010. Characterization of electrical penetration graphs of the Asian citrus psyllid, *Diaphorina citri*, in sweet orange seedlings. *Entomologia Experimentalis et Applicata* 134(1):35–49.

Bonavia, E. 1888. The Cultivated Oranges and Lemons, etc. of India and Ceylon, With Researches into their Origin and the Derivation of their Names, and Other Useful Information, with an Atlas of Illustrations. London: W.H. Allen.

Bond, J.E., and M.L. Roose. 1998. *Agrobacterium* mediated transformation of the commercially important citrus cultivar Washington navel orange. *Plant Cell Reports* 18(3-4):229–234.

Bové, J.M. 2006. Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology* 88(1):7–37.

———. 2009. Citrus diseases, huanglongbing, text and image gallery. 5 June, 2006. Riverside, CA: International Organization of Citrus Virologists. Available online at http://www.ivia.es/iocv/enfermedades/huanglongbing/fotos/arles_gallery/index.html. Accessed October 12, 2009.

Bové, J.M. 2009. History, distribution and importance. (January 2009 Revision). February 13, 2009. Villenave d'Ornon, France: International Organization of Citrus Virologists. Available online at http://www.ivia.es/iocv/enfermedades/huanglongbing/HUANGLONGBING.htm. Accessed August 6, 2009.

Bové, J.M., and M. Garnier. 1984. Citrus greening and psylla vectors of the disease in the Arabian Peninsula. In Proceedings of the 9th Conference of International Organization of Citrus Virologists, eds. S.M. Garnsey, L.W. Timmer and J.A. Dodds, pp. 109–114, May 9–13, 1983, at Argentina. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsIX/9th109_114.pdf. Accessed October 31, 2009.

———. 2003. Phloem-and xylem-restricted plant pathogenic bacteria. *Plant Science* 164(3):423–438.

Bové, J.M., E.C. Calavan, S.P. Capoor, R.E. Cortez, and R.E. Schwarz. 1974. Influence of temperature on symptoms of California stubborn, South African greening, Indian citrus decline, and Philippines leaf mottling diseases. In Proceedings of the 6th Conference of International Organization of Citrus Virologists, eds. L.G. Weathers and M. Cohen, pp. 12–15, August 21–28, at Swaziland. Riverside, CA: IOCV.

Bové, J.M., P. Bonnet, M. Gamier, and B. Aubert. 1980. Penicillin and tetracycline treatments of greening disease-affected citrus plants in the glasshouse, and the bacterial nature of the prokaryote associated with greening. In Proceedings of the 8th Conference of the International Organization of Citrus Virologists, eds. E.C. Calavan, S.M. Garnsey and L.W. Timmer, pp. 91–102, May 13–31, 1979, at Australia. Riverside, CA: IOCV.

Bové, J.M., M. Garnier, Y.S. Ahlawat, N.K. Chakraborty, and A. Varma. 1993. Detection of the Asian Strains of the greening BLO by DNA-DNA hybridization in Indian orchard trees and Malaysian *Diaphorina citri* psyllids. In Proceedings of the 12th Conference of the International Organization of Citrus Virologists, eds. P. Moreno, J.V. da Graça and L.W. Timmer, pp. 258–263, November 23–27, 1992, at New Delhi, India. Riverside, CA: IOCV.

Bové, J.M., M.E. Dwiastuti, A. Triviratno, A. Supriyanto, P.B. E. Nasli, and M. Garnier. 2000a. Incidence of huanglongbing and citrus rehabilitation in North Bali, Indonesia. In Proceedings of the 14th Conference of the International Organization of Citrus Virologists, eds. J.V. da Graça, R.F. Lee and R.K. Yokomi, pp. 200–206, May 13–31, IOCV, Riverside, CA. Available online at http://www.ivia.es/iocv/archivos/ProceedingsXV/Proceedings%20XIV%20Conference/200-206.pdf. Accessed October 31, 2009.

Bové, J.M., J.L. Danet, K. Bananej, N. Hassanzadeh, M. Taghizadeh, M. Salehi, and M. Garnier. 2000b. Witches' broom disease of lime (WBDL) in Iran. In Proceedings of the 14th Conference of the International Organization of Citrus Virologists, eds. J.V. da Graça, R.F. Lee and R.K. Yokomi, pp. 207–212, May 13–31, IOCV, Riverside, CA. Available online at http://www.ivia.es/iocv/archivos/ProceedingsXV/Proceedings%20XIV%20Conference/207-212.pdf. Accessed October 31, 2009.

Brlansky, R.H., and M.E. Rogers. 2007. Citrus huanglongbing: Understanding the vector-pathogen interaction for disease management. St. Paul, MN: American Phytopathological Society. Available online at http://www.apsnet.org/online/feature/HLB/. Accessed December 30, 2009.

Brlansky, R.H., M.M. Dewdney, M.E. Rogers, and K.R. Chung. 2009. Huanglongbing (citrus greening). 2009 Florida Citrus Pest Management Guide. Publication #PP-225. Gainesville: Institute of Food and Agricultural Sciences, University of Florida. Available online at http://edis.ifas.ufl.edu/CG086. Accessed February 2, 2010.

Brown, M.G. 1995. Florida and Brazil orange-juice trends. July 26, 1995. Gainesville: University of Florida. Available online at http://www.floridajuice.ifas.ufl.edu/pubs/misc/trend795.html. Accessed August 4, 2009.

Brown, M. G., T. H. Spreen, and J.-Y. Lee. 2004. Impacts on U.S. prices of reducing orange juice tariffs in major world markets. *Journal of Food Distribution Research* 35(2):26–33.

Brown, J.E., J.M. Dangler, F.M. Woods, K.M. Tilt, M.D. Henshaw, W.A. Griffey, and M.S. West. 1993. Delay in mosaic virus onset and aphid vector reduction in summer squash grown on reflective mulches. *HortScience* 28(9):895-896.

Buitendag, C.H., and L.A. von Broembsen. 1993. Living with citrus greening in South Africa. In Proceedings of the 12th Conference of the International Organization of Citrus

Virologists, eds. P. Moreno, J.V. da Graça and L.W. Timmer, pp. 269–273, November 23–27, 1992, at New Delhi, India. Riverside, CA: IOCV.

Burckhardt, D., and M. Martinez. 1989. Note sur la presence au Honduras d'un redoutable ennemi des citrus: *Diaphorina citri* Kuwayama (Hom. Psylloidea Psyllidae). *Bulletin de la Société Entomologique de France* 94:65–66.

Bushlad, R.C., and D.E. Hopkin. 1953. Sterilization of screw-worm flies with X-rays and gamma-rays. *Journal of Economic Entomology* 46(4):648–656.

Canny, G., and O. Levy. 2008. Bactericidal/permeability-increasing protein (BPI) and BPI homologs at mucosal sites. *Trends in Immunology* 29(11):541-547.

Capoor, S.P. 1963. Decline of citrus trees in India. *Bulletin, National Institute of Science India* 34(24):48–64.

Capoor, S.P., D.G. Rao, and S.M. Viswanath. 1967. *Diaphorina citri* Kuway., a vector of greening disease of citrus in India. *Indian Journal of Agricultural Science* 37:572–576.

———. 1974. Greening Disease of Citrus in the Deccan Trap Country and its Relationship with the Vector, *Diaphorina citri* Kuwayama. In Proceedings of the 6th Conference of International Organization of Citrus Virologists, eds. L.G. Weathers and M. Cohen, pp. 43–49, August 21–28, at Swaziland. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedings/6/6th043_049.pdf. Accessed September 8, 2009.

Cardoza, Y.J., and J.H. Tumlinson. 2006. Compatible and incompatible *Xanthomonas* infections differentially affect herbivore-induced volatile emission by pepper plants. *Journal of Chemical Ecology* 32(8):1755–1768.

Carter, D.J., and R.B. Cary. 2007. Lateral flow microarrays: A novel platform for rapid nucleic acid detection based on miniaturized lateral flow chromatography. *Nucleic Acids Research* 35(10):e74; doi:10.1093/nar/gkm269.

Catling, H.D. 1969. The bionomics of the South African Citrus Psylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae). The influence of extreme of weather on survival. *Journal of the Entomological Society of Southern Africa* 32(1969):237–290.

Catling, H.D., and P.R. Atkinson. 1974. Spread of greening by *Trioza erytreae* (Del Guercio) in Swaziland. In Proceedings of the 6th Conference of International Organization of Citrus Virologists, eds. L.G. Weathers and M. Cohen, pp. 33–39, August 21–28, at Swaziland. Riverside, CA: IOCV.

Caudwell, A. 1984. A compilation of the ten years work conducted in the laboratory of mycoplasmas at INRA Dijon to solve the riddle of grapevine flavescence dorée and other yellow's diseases of plants (1969–1981). Dijon, France: INRA.

CCQC (California Citrus Quality Council). 2010. Chemical Advisory. January 5, 2010. Auburn, CA: California Citrus Quality Council. Available online at http://www.calcitrusquality.org/wp-content/uploads/2009/05/Advisory-Spirotetramat-15101.pdf. Accessed March 4, 2010.

CDFA (California Department of Food and Agriculture). 2009. Asian citrus psyllid (ACP) and huanglongbing (HLB). Sacramento: California Department of Food and Agriculture. Available online at http://www.cdfa.ca.gov/phpps/acp/. Accessed Novermber 10, 2009.

———. 2009. *Pierce's disease: A decade of progress*. Sacramento, CA: CDFA Pierce's Disease Control Program. Available online at http://www.cdfa.ca.gov/pdcp/images/PD_GWSS_Decade_of_Progress_Brochure1.pdf. Accessed January 30, 2010.

Cermeli, M., P. Morales, and P. Godoy. 2000. Presencia del psilido asiatico de los citricos *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) en Venezuela. *Boletin de Entomologia Venezolana* 15:235–243.

Cernadas, R.A., L.R. Camillo, and C.E. Benedetti. 2008. Transcriptional analysis of the sweet orange interaction with the citrus canker pathogens *Xanthomonas axonopodis* pv. *citri* and *Xanthomonas axonopodis* pv. *aurantifolii*. *Molecular Plant Pathology* 9(5):609-631.

Cervera, M. 2005. Histochemical and fluorometric assays for uidA (GUS) gene detection. *Methods in molecular biology* 286:203–214.

Cervera, M., J. Juarez, A. Navarro, J.A. Pina, N. Duran-Vila, L. Navarro, and L. Peña. 1998. Genetic transformation and regeneration of mature tissues of woody fruit plants bypassing the juvenile stage. *Transgenic Research* 7(1):51–59.

Cervera, M., C. Ortega, A. Navarro, L. Navarro, and L. Peña. 2000. Generation of transgenic citrus plants with the tolerance-to-salinity gene HAL2 from yeast. *Journal of Horticultural Science & Biotechnology* 75(1):26–30.

Chakraborti, D., A. Sarkar, H. Mondal, and S. Das. 2009. Tissue specific expression of potent insecticidal, *Allium sativum* leaf agglutinin (ASAL) in important pulse crop, chickpea (*Cicer arietinum* L.) to resist the phloem feeding *Aphis craccivora*. *Transgenic Research* 18(4):529–544.

Chang, C.J., M. Garnier, L. Zreik, V. Rossetti, and J.M. Bové. 1993. Culture and serological detection of the xylem-limited bacterium causing citrus variegated chlorosis and its identification as a strain of *Xylella fastidiosa*. *Current Microbiology* 27(3):137–142.

Chavan, V.M., and A.S. Summanwar. 1993. Population dynamics and aspects of the biology of citrus psylla, *Diaphorina citri* Kuwayama, in Maharashtra. In Proceedings of the 12th Conference of the International Organization of Citrus Virologists, eds. P. Moreno, J.V. da Graça and L.W. Timmer, pp. 286–290, November 23–27, 1992, at New Delhi, India. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsXII/12th286_290.pdf. Accessed June 10, 2009.

Chen, J., X. Pu, X. Deng, S. Liu, H. Li, and E. Civerolo. 2009. A Phytoplasma Related to '*Candidatus* Phytoplasma asteri' Detected in Citrus Showing Huanglongbing (Yellow Shoot Disease) Symptoms in Guangdong, P. R. China. *Phytopathology* 99(3):236–242.

Chiu, R.J., M.-Y. Tsai, and C.-H. Huang. 1979. Distribution and retention of tetracyclines in healthy and likubin-infected citrus trees following trunk transfusion. In, Vol. 1 - NSC symposium series, Proceedings of the R.O.C. - United States Cooperative Science Seminar on mycoplasma diseases of plants, eds. H.-J. Su and R.E. McCoy, pp. 143–152, n.d., at Taipei, Taiwan. National Science Council.

Ciccarone, A. 1957. Il "giallume" ("yellow shoot") degli agrume in Cina. *Rivista di Agrumicoltura* 2:45–50.

Cirvilleri, G., S. Spina, G. Scuderi, A. Gentile, and A. Catara. 2005. Characterization of antagonistic root-associated fluorescent pseudomonads of transgenic and non-transgenic citrange Troyer plants. *Journal of Plant Pathology* 87(3):179–186.

Civerolo, E.L. 1984. Bacterial canker disease of citrus. *Journal of the Rio Grande Valley Horticultural Society* 37:127–146.

Cocco, A., and M.A. Hoy. 2009. Toxicity of organosilicone adjuvants and selected pesticides to the Asian citrus psyllid (Hemiptera: Psyllidae) and its parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae). *Florida Entomologist* 91(4):610-620.

Cocroft, R.B. 2001. Vibrational communication and the ecology of group-living, herbivorous insects. *American Zoologist* 4:1215–1221.

Coletta-Filho, H.D., M.L.P.N. Targon, M.A. Takita, J.D. De Negri, J. Pompeu, M.A. Machado, A.M. do Amaral, and G.W. Muller. 2004. First report of the causal agent of huanglongbing (*Candidatus* Liberibacter asiaticus) in Brazil. *Plant Disease* 88(12):1382–1382.

Coletta-Filho, H., F.H. Pereira Junior, C. Ferreira, and J.R. Spotti Lopes. 2009. Acquisition of *Candidatus* Liberibacter asiaticus by *Diaphorina citri* from sweet orange plants with variable pathogen population; Poster 10. Paper presented at the Citrus HLB and Potato ZC Conference: Status of Diseases and Research Opportunities, November, in McAllen, TX.

Conant, P., C. Hirayama, B.R. Kumashiro, R.A. Heu, and C.L. Young. 2007. Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) Updated February 2, 2009. No. 06-01. Honolulu, HI: State of Hawaii Department of Agriculture. Available online at http://hawaii.gov/hdoa/pi/ppc/npa-1/npa06-01-ACP.pdf. Accessed July 31, 2009.

Conesa, C., C. Rota, E. Castillo, M. D. Perez, M. Calvo, Aacute, and L. Sanchez. 2009. Antibacterial activity of recombinant human lactoferrin from rice: Effect of heat treatment. *Bioscience, Biotechnology, and Biochemistry* 73(6):1301-1307.

Costa, A.S., and G.W. Müller. 1980. Tristeza control by cross protection: A U.S.-Brazil cooperative success. *Plant Disease* 64(6):538–541.

Costa, M., W. Otoni, and G. Moore. 2002. An evaluation of factors affecting the efficiency of *Agrobacterium*-mediated transformation of *Citrus paradisi* (Macf.) and production of transgenic plants containing carotenoid biosynthetic genes. *Plant Cell Reports* 21(4):365–373.

Cunniffe, N.J., and C.A. Gilligan. 2010. Invasion, persistence and control in epidemic models for plant pathogens: the effect of host demography. *Journal of The Royal Society Interface* 7(44):439-451.

da Graça, J.V. 1991. Citrus greening disease. *Annual Review of Phytopathology* 29:109–136.

da Graça, J.V., and L. Korsten. 2004. Citrus huanglongbing: Review, present status and future strategies. In *Diseases of Fruits and Vegetables*, Vol. 1. ed. S.H. Naqvi. pp. 229–245 Dordrecht, The Netherlands: Kluwer Academic Publishers.

Dandekar, A.M., C. Davis, O. Fiehn, and R. D'Souza. 2009. A generalized reagentless sensor to detect citrus plant and fruit responses: Progress report FY 2008-2009. Visalias: California Citrus Research Board. Available online at http://citrus_static .hivip.org/reports/2008/09/25/dandekar-sept-08.pdf. Accessed November 3, 2009.

Davis, C.E., M.J. Bogan, S. Sankaran, M.A. Molina, B.R. Loyola, Z. Weixiang, W.H. Benner, M. Schivo, G.R. Farquar, N.J. Kenyon, and M. Frank. 2010. Analysis of volatile and non-volatile biomarkers in human breath using differential mobility spectrometry (DMS). *IEEE Sensors Journal* 10(1):114-122.

Davis, M.J., S.N. Mondal, H. Chen, M.E. Rogers, and R.H. Brlansky. 2008. Co-cultivation of "*Candidatus* Liberibacter asiaticus" with *Actinobacteria* from citrus with huanglongbing. *Plant Disease* 92(11):1547–1550.

Dawson, G.W., D.C. Griffiths, L.A. Merritt, A. Mudd, J.A. Pickett, L.J. Wadhams, and C.M. Woodcock. 1990. Aphid semiochemicals—A review and recent advances on the sex pheromone. *Journal of Chemical Ecology* 16:3019–3030.

Dawson, W.O., A.S. Folimonov, S.Y. Folimonova, M. Dekkers, A. Redondo, and J.H. Graham. 2005. Rapid screening of anti-bacterial gene products in citrus trees using a CTV-based transient expression vector. In Proceedings of the 2nd International citrus canker and huanglongbing research workshop, eds. T.R. Gottwald, W.N. Dixon, J.H. Graham and P. Berger, November 7–11, at Orlando, FL. Florida Citrus Mutual/ Florida Department of Agriculture and Consumer Services. Available online at http://www.doacs.state.fl.us/pi/chrp/images/ 2nd_International_Canker_Huanglongbing_Research_Workshop_2005.pdf. Accessed February 23, 2009.

De Moraes, C.M., J.C. Schultz, M.C. Mescher, and J.H. Tumlinson. 2004. Induced plant signaling and its implications for environmental sensing. *Journal of Toxicology and Environmental Health, Part A: Current Issues* 67(8):819–834.

Derrick, K.S., and L.W. Timmer. 2000. Citrus blight and other diseases of recalcitrant etiology. *Annual Review of Phytopathology* 38:181–205.

Dewdney, M., and J. Graham. 2009. Cooperative producers keep greening disease under control. *Citrus Industry*, September: 5–8.

Ding, F., G. Wang, G. Yi, Y. Zhong, J. Zeng, and B. Zhou. 2005. Infection of wampee and lemon by the citrus huanglongbing pathogen (*Candidatus* Liberibacter asiaticus) in China. *Journal of Plant Pathology* 87(3):207–212.

Doi, Y., M. Teranaka, K. Yora, and H. Asuyama. 1967. Mycoplasma-or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom. *Annals of the Phytopathological Society of Japan* 33(4):259–266.

Domínguez, A., J. Guerri, M. Cambra, L. Navarro, P. Moreno, and L. Peña. 2000. Efficient production of transgenic citrus plants expressing the coat protein gene of *Citrus tristeza* virus. *Plant Cell Reports* 19(4):427–433.

Down, R.E., E.C. Fitches, D.P. Wiles, P. Corti, H.A. Bell, J.A. Gatehouse, and J.P. Edwards. 2006. Insecticidal spider venom toxin fused to snowdrop lectin is toxic to the peachpotato aphid, *Myzus persicae* Hemiptera: Aphididae and the rice brown planthopper, *Nilaparvata lugens* Hemiptera: Delphacidae. *Pest Management Science* 62(1):77-85.

Driskell, J.D., O.M. Primera-Pedrozo, R.A. Dluhy, Y. Zhao, and R.A. Tripp. 2009. Quantitative surface-enhanced raman spectroscopy based analysis of microRNA mixtures. *Applied Spectroscopy* 63(10):1107-1114.

Du, Q., J.V. French, M. Skaria, C. Yang, and J.H. Everitt. 2004. Citrus pest stress monitoring using airborne hyperspectral imagery. In Proceedings of the 2004 International Geoscience and Remote Sensing Symposium (IGARSS), Vol. 6, pp. 3981–3984, September 20–24, at Anchorage, AK. IEEE Geoscience and Remote Sensing Society. Available online at http://www.scopus.com/inward/record.url ?eid=2-s2.0-15944366530&partnerID=40. Accessed January 24, 2009.

Du, Q., N.B. Chang, C. Yang, and K.R. Srilakshmi. 2008. Combination of multispectral remote sensing, variable rate technology and environmental modeling for citrus pest management. *Journal of Environmental Management* 86(1):14–26.

Duan, Y.P., T. Gottwald, L.J. Zhou, and D.W. Gabriel. 2008. First report of dodder transmission of "*Candidatus* Liberibacter asiaticus" to tomato (*Lycopersicon esculentum*). *Plant Disease* 92(5):831.

Duan, Y.P., L. Zhou, D.G. Hall, W. Li, H. Doddapaneni, H. Lin, L. Liu, C.M. Vahling, D.W. Gabriel, K.P. Williams, A. Dickerman, Y. Sun, and T. Gottwald. 2009. Complete genome sequence of citrus huanglongbing bacterium, "*Candidatus* Liberibacter asiaticus" obtained through metagenomics. *Molecular Plant-Microbe Interactions* 22(8):1011–1020.

Dugravot, S., E. Thibout, A. Abo-Ghalia, and J. Huignard. 2004. How a specialist and a non-specialist insect cope with dimethyl disulfide produced by *Allium porrum*. *Entomologia Experimentalis et Applicata* 113(3):173–179.

Durrant, W.E., and X. Dong. 2004. Systemic acquired resistance. *Annual Review of Phytopathology* 42:185–209.

Endo, T., T. Shimada, H. Fujii, Y. Kobayashi, T. Araki, and M. Omura. 2005. Ectopic expression of an FT homolog from citrus confers an early flowering phenotype on trifoliate orange (*Poncirus trifoliata* L. Raf.). *Transgenic Research* 14(5):703–712.

EPPO/CABI (European and Mediterranean Plant Protection Organization). 1997. *Trioza erytreae* and citrus greening bacterium. In Quarantine Pests for Europe: Data Sheets on Quarantine Pests for the European Union and for the European and Mediterranean Plant Protection Organization. ed. I.M. Smith. pp. 547–550 and pp. 971–976. 2nd edition. Wallingford: CAB International in association with the European and Mediterranean Plant Protection Organization.

Étienne, J., D. Burckhardt, and C. Grapin. 1998. *Diaphorina citri* (Kuwayama) en Guadeloupe, premier signalement pour les Caraïbes (Hem., Psyllidae). *Bulletin de la Société Entomologique de France* 103(1):32.

Étienne, J., S. Quilici, D. Marival, and A. Franck. 2001. Biological control of *Diaphorina citri* (Hemiptera: Psyllidae) in Guadeloupe by imported *Tamarixia radiata* (Hymenoptera: Eulophidae). *Fruits* 56(5):307–315.

Etxeberria, E., P. Gonzalez, W. Dawson, and T. Spann. 2007. An iodine-based starch test to assist in selecting leaves for HLB testing. December 2007. HS1122. Gainesville: University of Florida/IFAS. Available online at http://edis.ifas.ufl.edu/hs375. Accessed October 20, 2009.

Etxeberria, E., P. Gonzalez, D. Achor, and G. Albrigo. 2009. Anatomical distribution of abnormally high levels of starch in HLB-affected Valencia orange trees. *Physiological and Molecular Plant Pathology* 74(1):76-83.

Faghihi, M.M., M. Salehi, A. Bagheri, and K. Izadpanah. 2009. First report of citrus huanglongbing disease on orange in Iran. *Plant Pathology* 58(4):793.

Falivene, S., G. Sanderson, and S. Hardy. 2003. Water use efficiency: Citrus. Mildura: Horticulture Australia Limited/Riverlink. Available online at http://www.horticulture.com.au/librarymanager/libs/108/Water%20Use%20Efficiency%20-%20Citrus.pdf. Accessed April 30, 2009.

FAOSTAT (Food and Agriculture Organization Corporate Statistical Database). 2007. Food and agricultural commodities production: Countries by commodity: Oranges. From UN-FAO, Available online at http://faostat.fao.org/site/339/default.aspx. Accessed August 12, 2009.

FDACS-DPI (Florida Department of Agriculture and Consumer Services-Division of Plant Industry). 2009. Citrus greening disease: Statewide positive citrus greening sections. (February edition). Gainesville, FL: Florida Department of Agriculture and Consumer Services/ Division of Plant Industry. Available online at http://www.doacs.state.fl.us/pi/chrp/greening/citrusgreening.html. Accessed September 23, 2009.

FDEP (Florida Department of Environmental Protection). 2009. Ground water program. Tallahassee: Florida Department of Environmental Protection. Available online at http://www.dep.state.fl.us/water/groundwater/index.htm. Accessed August 22, 2009.

Febres, V.J., C.L. Niblett, R.F. Lee, and G.A. Moore. 2003. Characterization of grapefruit plants (*Citrus paradisi* Macf) transformed with *Citrus tristeza* closterovirus genes. *Plant Cell Reports* 21(5):421–428.

Ferwerda, J.G. 2005. Charting the quality of forage: Measuring and mapping the variation of chemical components in foliage with hyperspectral remote sensing. Ph.D. dissertation. ITC, Wageningen University, Wageningen, 183 pp. Available online at http://www.library.wur.nl/wda/dissertations/dis3791.pdf. Accessed June 12, 2009.

FGDL (Florida Geographic Data Library). 2007. Water management districts land use and cover 2007 [various]. From Florida Geographic Data Library, Available online at http://www.fgdl.org/. Accessed September 17, 2009.

Florida Citrus Mutual. 2009. Citrus industry history. Lakeland, FL: Florida Citrus Mutual, Inc. Available online at http://www.flcitrusmutual.com/citrus-101/citrushistory.aspx. Accessed August 2, 2009.

FCPRAC (Florida Citrus Production Research Advisory Council). 2010. Quarterly and final reports: Control of citrus greening, canker and emerging diseases of citrus: Citrus genome sequencing has begun to provide tools in the fight against HLB. January 18. Gainesville: FCPRAC - Florida Citrus Advanced Technology Program. Available online at http://citrus_static.hivip.org/reports/2010/01/18/ FCPRAC_ICGC_quarterly_report_1-10.pdf. Accessed March 9, 2010.

Folimonova, A.S., S.Y. Folimonova, M. Bar-Joseph, and W.O. Dawson. 2007. A stable RNA virus-based vector for citrus trees. *Virology* 368(1) Nov 10:205–216.

Folimonova, S.Y., C.J. Robertson, S.M. Garnsey, S. Gowda, and W.O. Dawson. 2009. Examination of the responses of different genotypes of citrus to huanglongbing (citrus greening) under different conditions. *Phytopathology* 99(12):1346-1354.

Follmer, C. 2008. Insights into the role and structure of plant ureases. *Phytochemistry* 69(1):18-28.

Forrest, E.D., and J.G. Chris. 1986. Aldicarb studies in ground waters from Florida citrus groves and their relation to ground water protection. *Ground Water* 24(1):16–22.

Forrester, N.W., M. Cahill, L.J. Bird, and J.K. Layland. 1993. Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. *Bulletin of Entomological Research* 1(Suppl. 1):1–132.

Francis, M.I., A. Redondo, J.K. Burns, and J.H. Graham. 2009. Soil application of imidacloprid and related SAR-inducing compounds produces effective and persistent control of citrus canker. *European Journal of Plant Pathology* 124(2):283–291.

Fraser, L.R., and D. Singh. 1968. Citrus dieback in India: The contribution of greening virus. In Proceedings of the 4th Conference of International Organization of Citrus Virologists, ed. J.F.L. Childs, pp. 141–144, October 2–12, 1966, at Catania, Italy. Gainesville: University of Florida Press.

French, J.V., C.J. Kahlke, and J.V.d. Graça. 2001. First record of the Asian citrus psylla, *Diaphorina citri* Kuwayama (Homoptera:Psyllidae), in Texas. *Subtropical Plant Science* 53:14–15.

Fu, G.L., K.C. Condon, M.J. Epton, P. Gong, L. Jin, G.C. Condon, N.I. Morrison, T.H. Dafa'alla, and L. Alphey. 2007. Female-specific insect lethality engineered using alternative splicing. *Nature Biotechnology* 25(3):353–357.

Fung, Y.-C., and C.-N. Chen. 2006. Effects of temperature and host plant on population parameters of the citrus psyllid (*Diaphorina citri* Kuwayama). *Formosan Entomology* 26(2006):109–123.

Gabriel, D.W., and S. Zhang. 2009. Two separate phage genomes appear associated with citrus greening (huanglongbing). Abstract supplement. *Phytopathology* 99(6):S38–39.

Gao, S., M. Garnier, and J.M. Bové. 1993. Production of monoclonal antibodies recognizing most Asian strains of the greening BLO by in vitro immunization with an antigenic protein purified from the BLO. In Proceedings of the 12th Conference of the International Organization of Citrus Virologists, eds. P. Moreno, J.V. da Graça and L.W. Timmer, pp. 244–249, November 23–27, 1992, at New Delhi, India. Riverside, CA: IOCV.

Garnett, H.M. 1985. Isolation of the greening organism. *Citrus and Subtropical Fruit Journal* 611:4–6.

Garnier, M., and J.M. Bové. 1983. Transmission of the organism associated with citrus greening disease from sweet orange to periwinkle by dodder. *Phytopathology* 73(10):1358–1363.

———. 1993. Citrus greening disease and the greening bacterium. In Proceedings of the 12th Conference of the International Organization of Citrus Virologists, eds. P. Moreno, J.V. da Graça and L.W. Timmer, pp. 212–219, November 23–27, 1992, at New Delhi, India. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsXII/12th212_219.pdf. Accessed September 16, 2009.

———. 1996. Distribution of the huanglongbing (greening) liberobacter species in fifteen African and Asian countries. In Proceedings of the 13th Conference of the International Organization of Citrus Virologists, eds. P. Moreno, J.V. da Graça and L.W. Timmer, pp. 388–391, November 16–23,1995, at Fouzhou, China. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsXIII/13th388_391.pdf. Accessed September 16, 2009.

Garnier, M., N. Daniel, and J.M. Bové. 1984. Aetiology of citrus greening disease. *Annales de microbiologie* 135 A(1):169–179.

———. 1984b. The greening organism is a gram negative bacterium. In Proceedings of the 9th Conference of International Organization of Citrus Virologists, eds. S.M. Garnsey, L.W. Timmer and J.A. Dodds, pp. 115–124, May 9–13, 1983, at Argentina. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsIX/9th115_124.pdf. Accessed February 3, 2009.

Garnier, M., S.J. Gao, Y.L. He, S. Villechanoux, J. Gandar, and J.M. Bové. 1991. Study of the greening organism (GO) with monoclonal antibodies: Serological identification, morphology, serotypes, and purification of the GO. In Proceedings of the 11th Conference of the International Organization of Citrus Virologists, eds. R.H. Brlansky, R.F. Lee and L.W. Timmer, pp. 428–435, November 6–10, 1989, at Orlando, FL. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsXI/11th428_435.pdf. Accessed June 28, 2009.

Garnier, M., L. Zreik, and J.M. Bové. 1991. Witches'-broom, a lethal mycoplasmal disease of lime trees in the Sultanate of Oman and the United Arab Emirates. *Plant Disease* 75(6):546–551.

Garnier, M., S. Jagoueix, P. Toorawa, M. Grisoni, R. Mallessard, A. Dookun, S. Saumtally, J.C. Autrey, and J.M. Bové. 1996. Both huanglongbing (greening) liberobacter species are present in Mauritius and Reunion. In Proceedings of the 13th Conference of the International Organization of Citrus Virologists, eds. P. Moreno, J.V. da Graça and L.W. Timmer, pp. 392–394, November 16–23, 1995, at Fouzhou, China. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsXIII/13th392_394.pdf. Accessed August 2, 2009.

Garnier, M., S. Jagoueix-Eveillard, P.R. Cronje, H.F. Le Roux, and J.M. Bové. 2000a. Genomic characterization of a Liberibacter present in an ornamental rutaceous tree, *Calodendrum capense*, in the Western Cape province of South Africa. Proposal of "*Candidatus* Liberibacter africanus subsp. capensis". *International Journal of Systematic and Evolutionary Microbiology* 50(6):2119–2125.

Garnier, M., J.M. Bové, C.P.R. Cronje, G.M. Sanders, L. Korsten, and H.F. Le Roux. 2000b. Presence of "*Candidatus* Liberibacter africanus" in the Western Cape province of South Africa. In Proceedings of the 14th Conference of the International Organization of Citrus Virologists, eds. J.V.d. Graça, R.F. Lee and R.K. Yokomi, pp. 369–372, Septermber 13–18, 1998, at Campinas, São Paulo. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/ProceedingsXV/Proceedings%20XIV%20Conference/369-372.pdf. Accessed February 16, 2009.

Garnsey, S.M., E.L. Civerolo, D.J. Gumpf, C. Paul, M.E. Hilf, R.F. Lee, R.H. Brlansky, R.K. Yokomi, and J.S. Hartung. 2005. Biological characterization of an international collection of *Citrus tristeza* virus (CTV) isolates. In, Vol. IOCV-2005, Proceedings of the 16th Conference of the International Organization of Citrus Virologists, eds. M.E. Hilf, N. Duran-Vila and M.A. Rocha-Peña, pp. 75–93, November 7–12, 2004, at Monterrey, Mexico. University of California. Available online at http://www.ivia.es/iocv/archivos/ProceedingsXV/proceedings%20XVI%20conference/075-93%20IOCV%2016.pdf. Accessed January 24, 2009.

Gatineau, F., H.T. Loc, N.D. Tuyen, T.M. Tuan, N.T.D. Hien, and N.T.N. Truc. 2006. Effects of two insecticide practices on population dynamics of *Diaphorina citri* and huanglongbing incidence in South Vietnam. In Proceedings of the Huanglongbing-Greening International Workshop, p. 110, July 16–20, at Ribeirão Preto, Brazil.

Gentile, A., S. Monticelli, and C. Damiano. 2002. Adventitious shoot regeneration in peach [*Prunus persica* (L.) Batsch]. *Plant Cell Reports* 20(11):1011–1016.

Ghanim, M., S. Kontsedalov, and H. Czosnek. 2007. Tissue-specific gene silencing by RNA interference in the whitefly *Bemisia tabaci* (Gennadius). *Insect Biochemistry and Molecular Biology* 37(7):732–738.

Ghorbel, R., A. Domínguez, L. Navarro, and L. Peña. 2000. High efficiency genetic transformation of sour orange (*Citrus aurantium*) and production of transgenic trees containing the coat protein gene of *Citrus tristeza* virus. *Tree Physiology* 20(17):1183–1189.

Golecki, B., A. Schulz, U. Carstens-Behrens, and R. Kollmann. 1998. Evidence for graft transmission of structural phloem proteins or their precursors in heterografts of Cucurbitaceae. *Planta* 206(4):630–640.

Gonsalves, D. 2004. Transgenic papaya in Hawaii and beyond. *AgBioForum* 7(1&2):36–40.

González Hernández, A.D. 2003. *Trioza erytreae* (Del Guercio, 1918): Nueva plaga de los cítricos en Canarias. *Phytoma España* 153:112–118.

González-Zamora, J.E., S. Camúñez, and C. Avilla. 2007. Effects of *Bacillus thuringiensis* cry toxins on developmental and reproductive characteristics of the predator *Orius albidipennis* (Hemiptera: Anthocoridae) under laboratory conditions. *Environmental Entomology* 36(5):1246-1253.

Gottwald, T.R., and M. Irey. 2007. Post-hurricane analysis of citrus canker II: Predictive model estimation of disease spread and area potentially impacted by various eradication protocols following catastrophic weather events. *Plant Health Progress* doi:10.1094/PHP-2007-0405-01-RS.

Gottwald, T.R., B. Aubert, and H.K. Long. 1991. Spatial pattern analysis of citrus greening in Shantou, China. In Proceedings of the 11th Conference of the International Organization of Citrus Virologists, eds. R.H. Brlansky, R.F. Lee and L.W. Timmer, pp. 421–427, November 6–10, 1989, at Orlando, FL. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsXI/11th421_427.pdf. Accessed November 6, 2009.

Gottwald, T.R., G. Hughes, J.H. Graham, X. Sun, and T. Riley. 2001. The citrus canker epidemic in Florida: The scientific basis of regulatory eradication policy for an invasive species. *Phytopathology* 91(1):30–34.

Gottwald, T.R., J.V. da Graça, and R.B. Bassanezi. 2007. Citrus huanglongbing: The pathogen and its impact. *Plant Health Progress* doi:10.1094/PHP-2007-0906-01-RV.

Gottwald, T.R., R.B. Bassanezi, L. Amorim, and A. Bergamin-Filho. 2007a. Spatial pattern analysis of citrus canker-infected plantings in São Paulo, Brazil, and augmentation of infection elicited by the Asian leafminer. *Phytopathology* 97(6):674–683.

Gottwald, T.R., M. Irey, T. Gast, S. Parnell, E. Taylor, and M.E. Hilf. 2009. Spatio-temporal analysis of an HLB epidemic in Florida and implications for future spread. In Proceedings of the 17th Conference of the International Organization of Citrus Virologists, p. 139, October 22–26, 2007, at Adana, Turkey. IOCV, Riverside, CA

Grafton-Cardwell, E., K.E. Godfrey, M.E. Rogers, C.C. Childers, and P.A. Stansly. 2006. Asian citrus psyllid. ANR Publication No. 206. Oakland: University of California. Accessed September 14, 2009.

Graham, J.H., and T.R. Gottwald. 1991. Research perspectives on eradication of citrus bacterial diseases in Florida. *Plant Disease* 75(12):1193–1200.

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

Grosser, J.W., P. Ollitrault, and O. Olivares-Fuster. 2000. Invited review: Somatic hybridization in citrus: An effective tool to facilitate variety improvement. *In Vitro Cellular and Developmental Biology - Plant* 36(6):434–449.

Growing Produce. 2009. Southern gardens citrus plants first commercial field trials of citrus trees resistant to canker and greening. June 23, 2009. Willoughby, OH: Meister Media

Worldwide. Available online at http://www.growingproduce.com/news/?storyid=2047. Accessed February 25, 2010.

Gudmestad, N.C., and G.A. Secor. 2007. Zebra chip: A new disease of potato. *Nebraska Potato Eyes*. University of Nebraska-Lincoln, Winter, 19 (Issue 1): 1–4. Available online at http://www.ndsu.edu/fileadmin/potatopathology/potato_trials/Zebra_Chip_New_Potato_Disease.pdf. Accessed November 18, 2009.

Guedot, C., D.R. Horton, and P.J. Landolt. 2008. Attraction of male winterform pear psylla to female-produced volatiles and to female extracts and evidence of male-male repellency. *Entomologia Experimentalis et Applicata* 130:191–197.

Guo, W.W., Y.X. Duan, O. Olivares-Fuster, Z.C. Wu, C.R. Arias, J.K. Burns, and J.W. Grosser. 2005. Protoplast transformation and regeneration of transgenic Valencia sweet orange plants containing a juice quality-related pectin methylesterase gene. *Plant Cell Reports* 24(8):482–486.

Gurr, G.M., S.L. Scarratt, S.D. Wratten, L. Berndt, and N. Irvin. 2004. Ecological engineering, habitat manipulation and pest management. In *Ecological Engineering for Pest Management: Advances in Habitat Manipulation for Arthropods*. eds. G. Gurr, S.D. Wratten and M.A. Altieri. pp. 1–12 Ithaca, N.Y.: Cornell University Press.

Gutiérrez-E, M.A., D. Luth, and G.A. Moore. 1997. Factors affecting *Agrobacterium*-mediated transformation in citrus and production of sour orange (*Citrus aurantium* L.) plants expressing the coat protein gene of *Citrus tristeza* virus. *Plant Cell Reports* 16(11):745–753.

Halbert, S.E. 2005. The discovery of huanglongbing in Florida. In, Vol. H-3 abstracts, Proceedings of the 2nd International Citrus Canker and Huanglongbing Research Workshop, p. 50, November 7–11, at Orlando, FL. Florida Citrus Mutual. Available online at http://www.doacs.state.fl.us/pi/chrp/images/2nd_International_Canker_Huanglongbing_Research_Workshop_2005.pdf. Accessed February 8, 2009.

Halbert, S.E., and K.L. Manjunath. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease in citrus: A literature review and assessment of risk in Florida. *Florida Entomologist* 87(3):330–353.

Halbert, S.E., and C.A. Núñez. 2004. Distribution of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Rhynchota: Psyllidae) in the Caribbean Basin. *Florida Entomologist* 87(3):401–402.

Halbert, S.E., H. Genc, B. Cevik, L.G. Brown, I.M. Rosales, K.L. Manjunath, M. Pomerinke, D.A. Davison, R.F. Lee, and C.L. Niblett. 2004. Distribution and characterization of *Citrus tristeza* virus in South Florida following establishment of *Toxoptera citricida*. *Plant Disease* 88(9):935–941.

Hall, D. 2008. Biology, history and world status of *Diaphorina citri*. In Proceedings of the North American Plant Protection Organization Workshop, Taller Internacional Sobre Huanglongbing y el Psilido Asiatico de los Citricos, May 7–9, at Hermosillo, Sonora. U.S. Department of Agriculture - APHIS. Available online at http://www.ars.usda.gov/research/publications/Publications.htm?seq_no_115=226004. Accessed November 2, 2009.

Hall, D.G., and L.G. Albrigo. 2007. Estimating the relative abundance of flush shoots in citrus with implications on monitoring insects associated with flush. *HortScience* 42(2):364–368.

Hall, D.G., M.G. Hent, and R.C. Adair, Jr. 2008. Population ecology and phenology of *Diaphorina citri* (Hemiptera: Psyllidae) in two Florida citrus groves. *Environmental Entomology* 37(4):914–924.

Hansen, A.K., J.T. Trumble, R. Stouthamer, and T.D. Paine. 2008. A new huanglongbing species, "*Candidatus* Liberibacter psyllaurous," found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). *Applied and Environmental Microbiology* 74(18):5862–5865.

Hao, L.Q., P. Yli-Pirilä, P. Tiitta, S. Romakkaniemi, P. Vaattovaara, M.K. Kajos, J. Rinne, J. Heijari, A. Kortelainen, P. Miettinen, J.H. Kroll, J.K. Holopainen, J.N. Smith, J. Joutsensaari, M. Kulmala, D.R. Worsnop, and A. Laaksonen. 2009. New particle formation from the oxidation of direct emissions of pine seedlings. *Atmospheric Chemistry and Physics* 9(20):8121-8137.

Hardy, S., G. Sanderson, P. Barkley, and N. Donovan. 2007. Dwarfing citrus trees using viroids. Primefact 704. New South Wales Department of Primary Industries.

Harrewijn, P., and H. Kayser. 1997. Pymetrozine, a fast-acting and selective inhibitor of aphid feeding. *In-situ* studies with electronic monitoring of feeding behaviour. *Pesticide Science* 49(2):130–140.

Hart, M.W., and R.K. Grosberg. 2009. Caterpillars did not evolve from Onychophorans by hybridogenesis. *Proceedings of the National Academy of Sciences* 106(47):19906–19909.

Hartung, J.S., J. Beretta, R.H. Brlansky, J. Spisso, and R.F. Lee. 1994. Citrus variegated chlorosis bacterium: Axenic culture, pathogenicity, and serological relationships with other strains of *Xylella fastidiosa*. *Phytopathology* 84(6):591–597.

Harvey, J.J.W., J.E. Lincoln, and D.G. Gilchrist. 2008. Programmed cell death suppression in transformed plant tissue by tomato cDNAs identified from an *Agrobacterium rhizogenes*-based functional screen. *Molecular Genetics and Genomics* 279(5):509–521.

Heppner, J.B. 1993. Citrus leafminer, *Phyllocnistis citrella*, in Florida (Lepidoptera: Gracillariidae: Phyllocnistinae). *Tropical Lepidoptera* 4(1):49–64.

Hidaka, T., M. Omura, M. Ugaki, M. Tomiyama, A. Kato, M. Ohsima, and F. Motoyoshi. 1990. *Agrobacterium*-mediated transformation and regeneration of *Citrus* spp. from suspension cells. *Japanese Journal of Breeding* 40(2):199–207.

Hilf, M.E., V.A. Mavrodieva, and S.M. Garnsey. 2005. Genetic marker analysis of a global collection of isolates of *Citrus tristeza* virus: Characterization and distribution of CTV genotypes and association with symptoms. *Phytopathology* 95(8):909–917.

Hocquellet, A., P. Toorawa, J.M. Bové, and M. Garnier. 1999. Detection and identification of the two *Candidatus* Liberibacter species associated with citrus huanglongbing by PCR amplification of ribosomal protein genes of theβ operon. *Molecular and Cellular Probes* 13(5):373–379.

Hölldobler, B., and E.O. Wilson. 1990. The Ants. 3rd ed. Cambridge, MA: Harvard University Press.

Holopainen, J.K. 2004. Multiple functions of inducible plant volatiles. *Trends in Plant Science* 9(11) Nov:529–533.

Hoshi, A., K. Oshima, S. Kakizawa, Y. Ishii, J. Ozeki, M. Hashimoto, K. Komatsu, S. Kagiwada, Y. Yamaji, and S. Namba. 2009. A unique virulence factor for proliferation and dwarfism in plants identified from a phytopathogenic bacterium. *Proceedings of the National Academy of Sciences* 106(15) April 14, 2009:6416-6421.

Hotz, R.L. 2009. The science prize: Innovation or stealth advertising? *The Wall Street Journal*, May 8, 2009. p. A9, Technology section, U.S. edition. Available online at http://online.wsj.com/article/SB124173078482897809.html. Accessed July 22, 2009.

Huang, C.H., M.Y. Tsai, and C.L. Wang. 1984. Transmission of citrus likubin by a psyllid, *Diaphorina citri*. *Journal of Agricultural Research of China* 33(1):65–72.

Huang, J., E.A. Schmelz, H. Alborn, J. Engelberth, and J.H. Tumlinson. 2005. Phytohormones mediate volatile emissions during the interaction of compatible and incompatible pathogens: The role of ethylene in *Pseudomonas syringae* infected tobacco. *Journal of Chemical Ecology* 31(3):439–459.

Huang, S., R. Li, Z. Zhang, L. Li, X. Gu, W. Fan, W.J. Lucas, X. Wang, B. Xie, P. Ni, Y. Ren, H. Zhu, J. Li, K. Lin, W. Jin, Z. Fei, G. Li, J. Staub, A. Kilian, E.A.G. van der Vossen, Y. Wu, J. Guo, J. He, Z. Jia, Y. Ren, G. Tian, Y. Lu, J. Ruan, W. Qian, M. Wang, Q. Huang, B. Li, Z. Xuan, J. Cao, Asan, Z. Wu, J. Zhang, Q. Cai, Y. Bai, B. Zhao, Y. Han, Y. Li, X. Li, S. Wang, Q. Shi, S. Liu, W.K. Cho, J.-Y. Kim, Y. Xu, K. Heller-Uszynska, H. Miao, Z. Cheng, S. Zhang, J. Wu, Y. Yang, H. Kang, M. Li, H. Liang, X. Ren, Z. Shi, M. Wen, M. Jian, H. Yang, G. Zhang, Z. Yang, R. Chen, S. Liu, J. Li, L. Ma, H. Liu, Y. Zhou, J. Zhao, X. Fang, G. Li, L. Fang, Y. Li, D. Liu, H. Zheng, Y. Zhang, N. Qin, Z. Li, G. Yang, S. Yang, L. Bolund, K. Kristiansen, H. Zheng, S. Li, X. Zhang, H. Yang, J. Wang, R. Sun, B. Zhang, S. Jiang, J. Wang, Y. Du, and S. Li. 2009. The genome of the cucumber, *Cucumis sativus* L. *Nature Genetics* 41, 1275-1281.

Hung, T.H., M.L. Wu, and H.J. Su. 2000. Identification of alternative hosts of the fastidious bacterium causing citrus greening disease. *Journal of Phytopathology* 148(6):321–326.

Hung, T.H., S.C. Hung, C.N. Chen, M.H. Hsu, and H.J. Su. 2004. Detection by PCR of *Candidatus* Liberibacter asiaticus, the bacterium causing citrus huanglongbing in vector psyllids: Application to the study of vector-pathogen relationships. *Plant Pathology* 53(1):96–102.

Hunter, W.B., S.E. Dowd, C.S. Katsar, R.G. Shatters, C.L. McKenzie, and D.G. Hall. 2009. Psyllid biology: Expressed genes in adult Asian citrus psyllids, *Diaphorina citri* Kuwayama. *Open Entomology Journal* 3(3):18-29.

Husain, M.A., and D. Nath. 1927. The citrus psylla (*Diaphorina citri*, Kuw.) (Psyllidae: Homoptera). In *Memoirs of the Department of Agriculture in India*, Vol. 10 pp. 1–27.

Hutson, S.S., N.L. Barber, J.F. Kenny, Kristin S. Linsey, D.S. Lumia, and M.A. Maupin. 2004. Estimated use of water in the United States in 2000. Circular 1268. Reston: U.S. Geological Survey. Available online at http://pubs.usgs.gov/circ/2004/circ1268/pdf/circular1268.pdf. Accessed May 19, 2009.

Hutton, R.J., and B.R. Cullis. 1981. Tree spacing effects on productivity of high density dwarf orange trees. *Proceedings of the International Society of Citriculture* 1:186–190.

Hutton, R., P. Broadbent, and K. Bevington. 2000. Viroid dwarfing for high density plantings. *Horticulture Reviews* 24:277–317.

IAGC (International Aphid Genomics Consortium). 2010. Genome Sequence of the Pea Aphid *Acyrthosiphon pisum*. *PLoS Biol*ogy 8(2): e1000313. doi:10.1371/journal.pbio.1000313.

IRAC (Insecticide Resistance Action Committee). 2009. *Mode of action classification*. version 2.7. CropLife International. 6.3 ed. Washington, DC: Insecticide Resistance Action Committee. Available online at http://www.irac-online.org/documents/pp_moa_structure_poster_v2.7_16oct09.pdf. Accessed January 30, 2010.

Irey, M.S., T. Gast, and T.R. Gottwald. 2006. Comparison of visual assessment and polymerase chain reaction assay testing to estimate the incidence of the Huanglongbing pathogen in commercial Florida citrus. *Proceedings of the Florida State Horticultural Society* 119(2006):89–93.

Irey, M., T.R. Gottwald, J.H. Graham, T.D. Riley, and G. Carlton. 2006a. Post-hurricane analysis of citrus canker spread and progress towards the development of a predictive model to estimate disease spread due to catastrophic weather events. *Plant Health Progress* (10.1094/PHP-2006-0822-01-RS).

Iwanami, T., T. Shimizu, T. Ito, and T. Hirabayashi. 2004. Tolerance to citrus mosaic virus in transgenic trifoliate orange lines harboring capsid polyprotein gene. *Plant Disease* 88(8):865–868.

Jagoueix, S., J.M. Bové, and M. Garnier. 1994. The phloem-limited bacterium of greening disease of citrus is a member of the α subdivision of the Proteobacteria. *International Journal of Systematic Bacteriology* 44(3):379–386.

———. 1996. PCR detection of the two '*Candidatus*' Liberobacter species associated with greening disease of citrus. *Molecular and Cellular Probes* 10(1):43–50.

———. 1997. Comparison of the 16S/23S ribosomal intergenic regions of *'Candidatus* Liberobacter asiaticum' and *'Candidatus* Liberobacter africanum,' the two species associated with citrus Huanglongbing (greening) disease. *International Journal of Systematic Bacteriology* 47(1):224–227.

Jones, R.L., and R.C. Back. 1984. Monitoring aldicarb residues in Florida soil and water. *Environmental Toxicology and Chemistry* 3(1):9–20.

Kalantidis, K., H.T. Schumacher, T. Alexiadis, and J.M. Helm. 2008. RNA silencing movement in plants. *Biology of the Cell* 100(1) Jan:13–26

Kaneyoshi, J., and S. Kobayashi. 1999. Characteristics of transgenic trifoliate orange (*Poncirus trifoliata* Raf.) possessing the rolC gene of *Agrobacterium rhizogenes* Ri plasmid. *Journal of the Japanese Society for Horticultural Science* 68(4):734–738.

Katz, M. 2008. Hydroponic systems, close spacings should bring profits to citrus growers sooner. *Citrus + Vegetable*, November 5, 2008, Available online at http://www.citrusandvegetable.com/PastIssues/ArticleLanding/tabid/70/Default.aspx?tid=1&cid=17335&issueid=654. Accessed September 9, 2009.

Kayim, M., T.L. Ceccardi, M.J.G. Berretta, G.A. Barthe, and K.S. Derrick. 2004. Introduction of a citrus blight-associated gene into carrizo citrange [*Citrus sinensis* (L.) Osbc. × *Poncirus trifoliata* (L.) Raf.] by *Agrobacterium*-mediated transformation. *Plant Cell Reports* 23(6):377–385.

Kiefer, W. 2008. Recent advances in linear and nonlinear Raman spectroscopy II. *Journal of Raman Spectroscopy* 39(12):1710-1725.

Kim, J.-S., U.S. Sagaram, J.K. Burns, J.-L. Li, and N. Wang. 2009. Response of sweet orange (*Citrus sinensis*) to *Candidatus* Liberibacter asiaticus infection: Microscopy and microarray analyses. *Phytopathology* 99(1):50–57.

Klueken, A.M., B. Hau, B. Ulber, and H.M. Poehling. 2009. Forecasting migration of cereal aphids (Hemiptera: Aphididae) in autumn and spring. *Journal of Applied Entomology* 133(5):328-344.

Knipling, E.F. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. *Journal of Economic Entomology* 48(4):459–462.

———. 1985. Sterile insect technique as a screwworm control measure: The concept and its development. Symposium on eradication of the screwworm from the United States and Mexico. *Miscellaneous Publications of the Entomological Society of America* 62:4–7.

Kobayashi, S., Y. Nakamura, J. Kaneyoshi, H. Higo, and K. Higo. 1996. Transformation of kiwifruit (*Actinidia chinensis*) and trifoliate orange (*Poncirus trifoliata*) with a synthetic gene encoding the human epidermal growth factor (hEGF). *Journal of the Japanese Society for Horticultural Science* 64(4):763–769.

Koizumi, M. 1985. Citrus canker, the world situation. In *Citrus Canker: An International Perspective*. ed. L.W. Timmer. pp. 2–7 Gainesville: Institute of Food and Agricultural Sciences, University of Florida.

Koltunow, A.M., A. Vivian-Smith, and S.R. Sykes. 2000. Molecular and conventional breeding strategies for seedless citrus. Abstract. *Acta Horticulturae (ISHS)* 535:169–174.

Krebs, M.D., A.M. Zapata, E.G. Nazarov, R.A. Miller, I.S. Costa, A.L. Sonenshein, and C.E. Davis. 2005. Detection of biological and chemical agents using differential mobility spectrometry (DMS) technology. *IEEE Sensors Journal* 5(4):696-703.

Kryder, R.D., S.P. Kowalski, and A.F. Krattiger. 2000. The intellectual and technical property components of pro-vitamin a rice (GoldenRice™): A preliminary freedom-to-operate review. ISAAA Briefs No. 20. Ithaca, NY: ISAAA Briefs. Available online at http://www.isaaa.org/resources/publications/briefs/20/ default.html. Accessed August 4, 2009.

Kumar, S., K. Chaudhary, J.M. Foster, J.F. Novelli, Y. Zhang, S. Wang, D. Spiro, E. Ghedin, and C.K. Carlow. 2007. Mining predicted essential genes of *Brugia malayi* for nematode drug targets. *PLoS One* 2(11) November 14:e1189.

Laflèche, D., and J.M. Bové. 1970a. Mycoplasmes dans les agrumes atteints de "greening", de "stubborn" ou de maladies similaires. *Fruits* 25:455–465.

———. 1970b. Structures de type mycoplasme dans les feuilles d'orangers atteints de la maladie du greening. *Comptes Rendus de l'Académie des sciences de Paris* 270:1915–1917.

Land and Water Australia. 2008. Impact of open hydroponics (OH) irrigation in the citrus industry. September 16th. Available online at http://lwa.gov.au/node/2632. Accessed 14th of October, 2009.

Landis, D.A., S.D. Wratten, and G.M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45:175–201.

Lankin-Vega, G., S.P. Worner, and D.A.J. Teulon. 2008. An ensemble model for predicting *Rhopalosiphum padi* abundance. *Entomologia Experimentalis et Applicata* 129(3):308-315.

Lanza, G., M.R. Calandra, M. Calvitti, C. Pedrotti, S. Barbagallo, M.E. Porto, and R. D'Anna. 2005. Evaluation of cold treatment against Mediterranean fruit fly in 'Tarocco' oranges. *Acta Horticulturae (ISHS)* 682:2141–2146.

Layden, L. 2009. Video/Photos: Some businesses find creative ways to combat deadly citrus disease known as greening. *Naplesnews.com*, News section. Available online at http://www.naplesnews.com/news/2009/aug/30/videophotos-some-businesses-find-creative-ways-com/#. Accessed December 12, 2009.

Lee, W.S., R. Ehsani, and L.G. Albrigo. 2008. Citrus greening (Huanglongbing) detection using aerial hyperspectral imaging. Paper presented at the 9th International Conference on Precision Agriculture, July 20–23, in Denver, CO. Fort Collins: Colorado State University/International

Plant Nutrition Institute/Foundation for Agronomic Research. Available online at http://www.icpaonline.org/. Accessed November 14, 2009.

Lemon, N., and A. Harless. 2008. USDA expands citrus greening and Asian citrus psyllid quarantine to portions of Louisiana. Washington, DC: U.S. Department of Agriculture - Animal and Plant Health Inspection Service. Available online at http://www.aphis.usda.gov/newsroom/content/2008/07/citrusgreening_acp_la.shtml. Accessed September 20, 2009.

Li, D.D., W. Shi, and X.X. Deng. 2002. *Agrobacterium*-mediated transformation of embryogenic calluses of ponkan mandarin and the regeneration of plants containing the chimeric ribonuclease gene. *Plant Cell Reports* 21(2):153–156.

Li, W., J.S. Hartung, and L. Levy. 2006. Quantitative real-time PCR for detection and identification of *Candidatus* Liberibacter species associated with citrus huanglongbing. *Journal of Microbiological Methods* 66:104–115.

———. 2007. Evaluation of DNA amplification methods for improved detection of "*Candidatus* Liberibacter species" associated with citrus huanglongbing. *Plant Disease* 91(1):51–58.

Li, Y.P., R.X. Xia, H. Wang, X.S. Li, Y.Q. Liu, Z.J. Wei, C. Lu, and Z.H. Xiang. 2009. Construction of a full-length cDNA Library from Chinese oak silkworm pupa and identification of a KK-42-binding protein gene in relation to pupa-diapause termination. *International Journal of Biological Sciences* 5(5):451–457.

Liefting, L.W., B.S. Weir, S.R. Pennycook, and G.R.G. Clover. 2009a. "*Candidatus* Liberibacter solanacearum", associated with plants in the family Solanaceae. *International Journal of Systematic and Evolutionary Microbiology* 59(9):2274–2276.

Liefting, L.W., P.W. Sutherland, L.I. Ward, K.L. Paice, B.S. Weir, and G.R.G. Clover. 2009b. A new "*Candidatus* Liberibacter" species associated with diseases of solanaceous crops. *Plant Disease* 93(3):208–214.

Lin, C.K. 1963. Notes on citrus yellow shoot disease. *Acta Phytophylactica Sinica* 2:243–251.

Lin, H., H. Doddapaneni, J.E. Munyaneza, E.L. Civerolo, V.G. Sengoda, J.L. Buchman, and D.C. Stenger. 2009. Molecular characterization and phylogenetic analysis of 16s rRNA from a new "*Candidatus* Liberibacter" strain associated with zebra chip disease of potato (*Solanum tuberosum* L.) and the potato psyllid (*Bactericera cockerelli* Sulc.). Short Communication. *Journal of Plant Pathology* 91(1):215–219.

Lin, K.H. 1956a. Observations on yellow shoot of citrus. *Acta Phytopathologica Sinica* 2:1–11.

———. 1956b. Etiological studies of yellow shoot of citrus. *Acta Phytopathologica Sinica* 2:13–42.

Lin, M.K., Y.J. Lee, T.J. Lough, B.S. Phimmey, and W.J. Lucas. 2009. Analysis of the pumpkin phloem proteome provides insights into angiosperm sieve tube function. *Molecular and Cellular Proteomics* 8(2):343–356.

Lindquist, A.W. 1955. The use of gamma radiation for control or eradication of the screw-worm. *Journal of Economic Entomology* 48:467–469.

Liu, Y.H., and J.H. Tsai. 2000. Effects of temperature on biology and life table parameters of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). *Annals of Applied Biology* 137(3):201–206.

Lopes, S.A., and G.F. Frare. 2008. Graft transmission and cultivar reaction of citrus to "*Candidatus* Liberibacter americanus". *Plant Disease* 92(1):21–24.

Lopes, S.A., E.C. Martins, and G.F. Frare. 2005. Deteção de *Candidatus* Liberibacter americanus em *Murraya paniculata*. *Summa Phytopathologica* 31:48–49.

———. 2006. Deteção de *Candidatus* Liberibacter asiaticus em *Murraya paniculata*. *Fitopatologia Brasileira* 31:303.

Lopes, S.A., G.F. Frare, P.T. Yamamoto, A.J. Ayres, and J.C. Barbosa. 2007. Ineffectiveness of pruning to control citrus huanglongbing caused by *Candidatus* Liberibacter americanus. *European Journal of Plant Pathology* 119(4):463–468.

Lopes, S.A., G.F. Frare, E. Bertolini, M. Cambra, N.G. Fernandes, A.J. Ayres, D.R. Marin, and J.M. Bové. 2009. Liberibacters associated with citrus huanglongbing in Brazil: "*Candidatus* Liberibacter asiaticus" is heat tolerant, "*Ca.* L. americanus" is heat sensitive. *Plant Disease* 93(3):257–262.

Lou, B.H., C.Y. Zhou, X.Y. Zhao, Z.-g. Li, M. Xu, J.X. Liu, Y. Zhou, and K.S. Tang. 2008. Primary study on species and intraspecific differentiations of HLB pathogens in eight provinces of China. In, Vol. P333 abstracts, Proceedings of the 11th International Citrus Congress - International Society of Citriculture, p. 232, October 26–30, at Wuhan, China. Gainesville: University of Florida Press.

Luft, P.A., T.D. Paine, and G.P. Walker. 2001. Interactions of colonization density and leaf environments on survival of *Trioza eugeniae*. *Ecological Entomology* 26:263–270.

Luis Pantoja, M., C. Collazo Cordero, R. Llauger Riveron, L. Peña Barzaga, D. Lopez Hernandez, E. Blanco, J.C. Casi, L. Batista Le Riverend, F.A.O. Tanaka, R.B. Salaroli, E. Martins, D. Teixeira, E. Kitajima, J. Ayres, J.M. Bové, J.L. Perez, and J.R. Cueto. 2008. Huanglongbing in Cuban citriculture. In Abstract 84: Proceedings of the 11th International Citrus Congress - International Society of Citriculture, pp. 50–51, October 26–30, at Wuhan, China. Gainesville: University of Florida Press.

Malenovsky, Z., K.B. Mishra, F. Zemek, U. Rascher, and L. Nedbal. 2009. Scientific and technical challenges in remote sensing of plant canopy reflectance and fluorescence. *Journal of Experimental Botany* 60(11):2987-3004.

Manjunath, K.L., S.E. Halbert, C. Ramadugu, S. Webb, and R.F. Lee. 2008. Detection of "*Candidatus* Liberibacter asiaticus" in *Diaphorina citri* and its importance in the management of citrus huanglongbing in Florida. *Phytopathology* 98(4):387–396.

Mao, J.Y., A.M. Belcher, and K.J.V. Vliet. 2010. Genetically engineered phage fibers and coatings for antibacterial applications. *Advanced Functional Materials* 20(2):209-214.

Mao, Y.-B., W.-J. Cai, J.-W. Wang, G.-J. Hong, X.-Y. Tao, L.-J. Wang, Y.-P. Huang, and X.-Y. Chen. 2007. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. 10.1038/nbt1352. *Nature Biotechnology* 25(11) 2007/11//print:1307–1313.

Martinez, A.L., and J.M. Wallace. 1967. Citrus leaf-mottle-yellows disease in the Philippines and transmission of the causal virus by a psyllid, *Diaphorina citri*. *Plant Disease Reporter* 51:692–695.

———. 1968. Studies on leaf-mottle-yellows disease of citrus in the Philippines. In Proceedings of the 4th Conference of International Organization of Citrus Virologists, pp. 167–176, at Catania, Italy. Gainesville: University of Florida Press. Available online at http://www.ivia.es/iocv/archivos/proceedings/4/4th167_176.pdf. Accessed August 6, 2009.

Marutani-Hert, M., W.B. Hunter, and D.G. Hall. 2009a. Establishment of Asian citrus psyllid (*Diaphorina citri*) primary cultures. *In Vitro Cellular and Developmental Biology - Animal*:1–4.

Marutani-Hert, M., W.B. Hunter, C.S. Katsar, X.H. Sinisterra, D.G. Hall, and C.A. Powell. 2009b. Reovirus-like sequences isolated from adult Asian citrus psyllid, (Hemiptera: Psyllidae: *Diaphorina citri*). *Florida Entomologist* 92(2):314–320.

Matos, L., M.E. Hilf, and J. Camejo. 2009. First report of "*Candidatus* Liberibacter asiaticus" associated with citrus huanglongbing in the Dominican Republic. *Plant Disease* 93(6):668–668.

McClean, A.P.D., and R.E. Schwarz. 1970. Greening of blotchy-mottle disease in citrus. *Phytophylactica* 2:177–194.

McClean, A.P.D., and P.C.J. Oberholzer. 1965a. Citrus psylla, a vector of the greening disease of sweet orange. *South Africa Journal of Agricultural Science* 8:297–298.

———. 1965b. Greening disease of sweet orange: Evidence that it is caused by a transmissible virus. *South Africa Journal of Agricultural Science* 8:253–276.

McCoy, C.W., M.E. Rogers, S.H. Futch, J.H. Graham, L.W. Duncan, and H.N. Nigg. 2009. Citrus root weevils. In *2009 Florida Citrus Pest Management Guide*. eds. M.E. Rogers, M.M. Dewdney and T.M. Spann. Gainesville: University of Florida/IFAS Extension.

McFarland, C.D., and M.A. Hoy. 2001. Survival of *Diaphorina citri* (Homoptera: Psyllidae), and its two parasitoids, *Tamarixia radiata* (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), under different relative humidities and temperature regimes. *Florida Entomologist* 84(2):227–233.

McKinsey and Company. 2009. "And the winner is ..." Capturing the promise of philanthropic prizes. New York: McKinsey and Company. Available online at http://www.mckinsey.com/clientservice/socialsector/And_the_winner_is.pdf. Accessed October 19, 2009.

Mead, F.W. 1998. Asian citrus psyllid, *Diaphorina citri* Kuwayama (Insecta: Hemiptera: Psyllidae). Featured Creatures. Revised: October 2009. EENY-033 (IN160). Gainesville: University of Florida. Available online at http://edis.ifas.ufl.edu/IN160. Accessed September 25, 2009.

Medina-Ortega, K.J., N.A. Bosque-Pérez, E. Ngumbi, E.S. Jiménez-Martínez, and S.D. Eigenbrode. 2009. *Rhopalosiphum padi* (Hemiptera: Aphididae) responses to volatile cues from barley yellow dwarf virus-infected wheat. *Environmental Entomology* 38(3):836-845.

Michaud, J.P. 2002. Biological control of Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae) in Florida: A preliminary report. *Entomological News* 113(3):216–222.

———. 2004. Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. *Biological Control* 29(2):260–269.

Miles, P.W. 1999. Aphid saliva. *Biological Reviews* 74(01):41–85.

Mishra, A.R., R. Ehsani, W.S. Lee, and G. Albrigo. 2007. Spectral characteristics of citrus greening (huanglongbing). Paper presented at the American Society of Agricultural and Biological Engineers Annual Meeting, June 17-20, in Minneapolis, MN. American Society of Agricultural and Biological Engineers. Available online at http://asae.frymulti.com/abstract.asp?aid=24163&t=2. Accessed October 30, 2009.

Mitchell, F.L., J. Brady, B. Bextine, and I. Lauzière. 2009. Seasonal increase of *Xylella fastidiosa* in Hemiptera collected from Central Texas vineyards. *Journal of Economic Entomology* 102(5):1743-1749.

MIT-TLO (Massachusetts Institute of Technology, Technology Licensing Office). 2006. Preserving your patent rights. August 18, 2009. Cambridge: Massachusetts Institute of

Technology. Available online at http://web.mit.edu/tlo/www/community/preserving_patent_rights.html. Accessed August 24, 2009.

Miyakawa, T., and K. Tsuno. 1989. Occurrence of citrus greening disease in southern islands of Japan. *Annals of the Phytopathological Society of Japan* 55:667–670.

Molina, M.A., W. Zhao, S. Sankaran, M. Schivo, N.J. Kenyon, and C.E. Davis. 2008. Design-of-experiment optimization of exhaled breath condensate analysis using a miniature differential mobility spectrometer (DMS). *Analytica Chimica Acta* 628(2) Nov 3:155–161.

Moll, J.N., and M.M. Martin. 1974. Comparison of the organism causing greening disease with several plant pathogenic Gram negative bacteria, rickettsia-like organisms and mycoplasma-like organisms. In Proceedings of the Conference Les Mycoplasmes/Mycoplasmas, Vol. 33 pp. 89–96, n.d., INSERM.

Moll, J.N., and S.P. van Vuuren. 1977. Greening disease in Africa. In Proceedings of the 2nd International Citrus Congress - International Society of Citriculture, Vol. 3, pp. 903–912, n.d., at Orlando, FL. Gainesville: University of Florida Press.

Moll, J.N., S.P.V. Vuuren, and D.L. Milne. 1980. Greening disease, the Southern African situation. In Proceedings of the 8th Conference of the International Organization of Citrus Virologists, eds. E.C. Calavan, S.M. Garnsey and L.W. Timmer, pp. 109–117, May 13–31, 1979, at Sydney, Australia. Riverside, CA: IOCV.

Moore, G.A. 2001. Oranges and lemons: Clues to the taxonomy of citrus from molecular markers. *Trends in Genetics* 17(9):536–540.

Moore, G.A., C.C. Jacono, J.L. Neidigh, S.D. Lawrence, and K. Cline. 1992. *Agrobacterium*-mediated transformation of Citrus stem segments and regeneration of transgenic plants. *Plant Cell Reports* 11(5-6):238–242.

Moore, G.A., V.J. Febres, C.L. Niblett, D. Luth, M. McCaffery, and S.M. Garnsey. 2000. *Agrobacterium*-mediated transformation of grapefruit (*Citrus Paradisi* Macf.) with genes from *Citrus tristeza* virus. *Acta Horticulturae (ISHS)* 535(29):237–243.

Morris, R.A., R.P. Muraro, and T.H. Spreen. 2008. Invasive diseases and fruit tree production: Economic tradeoffs of citrus greening control on Florida's citrus industry. Paper presented at the Southern Agricultural Economics Association: 2008 Annual Meeting, February 2–6, in Dallas, TX. Southern Agricultural Economics Association. Available online at http://purl.umn.edu/6309. Accessed November 14, 2009.

Morris, R.A., T.H. Spreen, and M.G. Brown. 2009. Short-term price effects resulting from high retail OJ prices and accumulated inventories. Lake Alfred, FL: Proceedings of the Florida State Horticultural Society. Available online at http://www.crec.ifas.ufl.edu/publications/submit/morris/morris_1.htm. Accessed November 8, 2009.

Muraro, R.P. 2008a. Summary of 2007-2008 citrus budget for the Indian River production region. Gainesville: University of Florida, Institute of Food and Agricultural Sciences. Available online at http://www.crec.ifas.ufl.edu/extension/economics/pdf/IR_Budget_Summ_2007-2008.pdf. Accessed August 23, 2009.

———. 2008b. Summary of 2007-2008 citrus budget for the Central Florida (Ridge) production region. Gainesville: University of Florida, Institute of Food and Agricultural Sciences. Available online at http://www.crec.ifas.ufl.edu/extension/economics/pdf/CF_Budget_Summ_2007_2008.pdf. Accessed October 13, 2009.

Murray, M.G., and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 8(19):4321–4325.

Murray, R.G.E., and K.H. Schleifer. 1994. Taxonomic notes: A proposal for recording the properties of putative taxa of procaryotes. *International Journal of Systematic Bacteriology* 44(1):174–176.

Mutti, N.S., Y. Park, J.C. Reese, and G.R. Reeck. 2006. RNAi knockdown of a salivary transcript leading to lethality in the pea aphid, *Acyrthosiphon pisum*. *Journal of Insect Science* 6(38):1–7.

Mutti, N.S., J. Louis, L.K. Pappan, K. Pappan, K. Begum, M.-S. Chen, Y. Park, N. Dittmer, J. Marshall, J.C. Reese, and G.R. Reeck. 2008. A protein from the salivary glands of the pea aphid, *Acyrthosiphon pisum*, is essential in feeding on a host plant. *Proceedings of the National Academy of Sciences* 105(29):9965–9969.

NAE (National Academy of Engineering; Steering Committee for the Workshop to Assess the Potential for Promoting Technological Advance through Government-Sponsored Prizes and Contests). 1999. Concerning Federally Sponsored Inducement Prizes in Engineering and Science. Washington, DC: National Academy Press.

Nakabachi, A., S. Koshikawa, T. Miura, and S. Miyagishima. 2009. Genome size of *Pachypsylla venusta* (Hemiptera: Psyllidae) and the ploidy of its bacteriocyte, the symbiotic host cell that harbors intracellular mutualistic bacteria with the smallest cellular genome. *Bulletin of Entomological Research* 99(1):1–7.

NAPPO (North American Plant Protection Organization). 2008. Phytosanitary alert system: Confirmation of Asian citrus psyllid, *Diaphorina citri*, in Alabama, Georgia, Mississippi, and South Carolina. August 28, 2008. Ottawa, Ontario: North American Plant Protection Organization. Available online at http://www.pestalert.org/oprDetail.cfm?oprID=340. Accessed November 02, 2009.

———. 2009a. Confirmation of citrus greening in Chatham County, Georgia - United States. June 22, 2009. Ottawa, Ontario: North American Plant Protection Organization. Available online at http://www.pestalert.org/oprDetail.cfm?oprID =382&keyword=HLB. Accessed December 15, 2009.

———. 2009b. First detection of Asian citrus psyllid *Diaphorina citri* in Arizona and new detection sites in California. December 16, 2009. Ottawa, Ontario: North American Plant Protection Organization. Available online at http://www.pestalert .org/oprDetail.cfm?oprID=411&keyword=Diaphorina%20citri. Accessed December 30, 2009.

———. 2009c. Expansion of Federal quarantine area for citrus greening due to the detection in the Commonwealth of Puerto Rico. November 23, 2009. Ottawa, Ontario: North American Plant Protection Organization. Available online at http://www.pestalert.org/oprDetail.cfm?oprID=407&keyword=Huanglongbing. Accessed December 30, 2009.

———. 2009d. Sistema de alerta fitosanitaria: Actualización de la detección de Huanglongbing (*Candidatus* Liberibacter asiaticus) en Árboles de traspatio en México. December 2009. Ottawa, Ontario: North American Plant Protection Organization. Available online at http://www.pestalert.org/espanol/oprDetail.cfm ?oprID=410&keyword=Diaphorina%20citri. Accessed December 30, 2009.

Nault, L.R., and P.L. Phelan. 1984. Alarm pheromones and sociality in pre-social insects. In *Chemical Ecology of Insects*, Vol. 2. eds. W.J. Bell and R.T. Cardé. pp. 237–256 Sunderland, MA: Sinauer Associates, Inc.

Nault, L.R., M.E. Montgomery, and W.S. Bowers. 1976. Ant aphid association: Role of aphid alarm pheromone. *Science* 192(4246):1349–1350.

Nava, D.E., M.L.G. Torres, M.D.L. Rodrigues, J.M.S. Bento, and J.R.P. Parra. 2007. Biology of *Diaphorina citri* (Hem., Psyllidae) on different hosts and at different temperatures. *Journal of Applied Entomology* 131(9-10):709–715.

NOAA-CSC (National Oceanic and Atmospheric Administration Coastal Services Center). 2009. Historical north atlantic tropical cyclone tracks, 1851-2008. from National Oceanic and Atmospheric Administration Coastal Services Center/Florida Geographic Data Library (FGDL), Available online at http://www.fgdl.org/download/index.html. Accessed August 3, 2009.

Norberg, R.P. 2008. Economic importance of Florida citrus. Paper presented at the U.S. Department of Agriculture-ARS 'SWAT Team' Workshop, April 22, in Ft. Pierce, FL. Florida Citrus Mutual, Inc. Available online at http://www.flcitrusmutual.com/files/e47fe5d8-ef81-4c15-9.pdf. Accessed May 13, 2009.

Norberg, R.P. 2009. Economic importance of Florida citrus. Paper presented at the Committee on Strategic Planning for the Florida Citrus Industry: Citrus Greening and the Future, May 15, in Washington, DC. National Research Council of The National Academies

Noronha, N.G., and J.M.S. Bento. 2008. Volatile of citrus and guava plantas: Something in the air to the psyllid *Diaphorina citri*. In, Vol. 1, Proceedings of the 25th Anniversary International Society of Chemical Ecology Meeting, p. 74, August 17–22, at State College, Pennsylvania. ISCE. Available online at http://chemecol.org/meetings/ISCE2008.pdf. Accessed March 12, 2009.

Novak, H. 1994. The influence of ant attendance on larval parasitism in hawthorn psyllids (Homoptera: Psyllidae). *Oecologia* 99:72–78.

NRC (National Research Council; Committee on the Design of an NSF Innovation Prize). 2007. Innovation Inducement Prizes: At the National Science Foundation. Washington, DC: National Academies Press.

Omoto, C., T.J. Dennehy, C.W. McCoy, S.E. Crane, and J.W. Long. 1995. Management of citrus rust mite (Acari: Eriophyidae) resistance to dicofol in Florida Citrus. *Journal of Economic Entomology* 88(5):1120–1128.

Opperman, C.H., D.M. Bird, V.M. Williamson, D.S. Rokhsar, M. Burke, J. Cohn, J. Cromer, S. Diener, J. Gajan, S. Graham, T.D. Houfek, Q. Liu, T. Mitros, J. Schaff, R. Schaffer, E. Scholl, B.R. Sosinski, V.P. Thomas, and E. Windham. 2008. Sequence and genetic map of *Meloidogyne hapla*: A compact nematode genome for plant parasitism. *Proceedings of the National Academy of Sciences of the United States of America* 105(39) Sep 30:14802–14807.

Osborne, L.S., and Z. Landa. 1992. Workshop on important arthropod pests of the Caribbean Basin amenable to biological control: *Homoptera*, *Coleoptera*, *Lepidoptera*: Biological control of whiteflies with entomopathogenic fungi. *Florida Entomologist* 75(4):456–471.

Panneton, B., S. Guillaume, J.-M. Roger, and G. Samson. 2010. Improved discrimination between monocotyledonous and dicotyledonous plants for weed control based on the blue-green region of ultraviolet-induced fluorescence spectra. *Applied Spectroscopy* 64(1):30-36.

Parsons, L.R., and K.M. Morgan. 2004. Management of microsprinkler systems for Florida citrus. Publ. No. HS-5958. Gainesville: University of Florida, IFAS.

Peña, L., and L. Navarro. 1999. Transgenic trees. In *Biotechnology in Agriculture and Forestry*. Vol. 44. ed. Y.P.S. Bajaj. pp. 39–54 Berlin; New York: Springer-Verlag.

Peña, L., M. Martin-Trillo, J. Juarez, J.A. Pina, L. Navarro, and J.M. Martinez-Zapater. 2001. Constitutive expression of *Arabidopsis LEAFY* or *APETALA1* genes in citrus reduces their generation time. *Nature Biotechnology* 19(3):263–267.

Percy, D.M. 2010. Psyllids. December 4, 2009. Vancouver, BC: University of British Columbia. Available online at http://www.psyllids.org/. Accessed January 30, 2010.

Pereira, J.L., L.C.C. De Almeida, and S.M. Santos. 1996. Witches' broom disease of cocoa in Bahia: Attempts at eradication and containment. *Crop Protection* 15(8):743–752.

Peres, N.A., and L.W. Timmer. 2003. Citrus Black Spot Caused by *Guignardia citricarpa*. Oxford, UK: CAB International.

Peres, N.A., L.W. Timmer, J.E. Adaskaveg, and J.C. Correll. 2005. Lifestyles of *Colletotrichum acutatum*. *Plant Disease* 89(8):784–796.

Piestun, D., O. Batuman, X. Che, R. Gofman, V. Filatov, S. Zypman, R. Gafny, and M. Bar-Joseph. 2000. Truncated versions of the *Citrus tristeza* virus (CTV) replicase and Basta resistance genes incorporated in transgenic Troyer citrange. *Acta Horticulturae (ISHS)* 535(Proceedings of the First International Symposium on Citrus Biotechnology, 1998):223–230.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. *BioScience* 50(1):53–65.

Planet, P., S. Jagoueix, J.M. Bové, and M. Garnier. 1995. Detection and characterization of the African citrus greening Liberobacter by amplification, cloning, and sequencing of the rplKAJL-rpoBC operon. *Current Microbiology* 30(3):137–141.

Poole, G., W. Windham, G. Heitschmidt, B. Park, and T. Gottwald. 2008. Visible/near-infrared spectroscopy for discrimination of HLB-infected citrus leaves from healthy leaves. In Proceedings of the International Research Conference on Huanglongbing, pp. 155–157, December 1–4, 2008, at Orlando, FL. Gainesville: University of Florida. Available online at http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=236390. Accessed February 26, 2010.

Prassinos, C., J.-H. Ko, G. Lang, A.F. Iezzoni, and K.-H. Han. 2009. Rootstock-induced dwarfing in cherries is caused by differential cessation of terminal meristem growth and is triggered by rootstock-specific gene regulation. *Tree Physiology* 29(7):927–936.

Price, P.W. 1984. Insect Ecology. 2nd ed. New York: Wiley.

Pridgeon, J.W., L.M. Zhao, J.J. Becnel, D.A. Strickman, G.G. Clark, and K.J. Linthicum. 2008. Topically applied AaeIAP1 double-stranded RNA kills female adults of *Aedes aegypti*. *Journal of Medical Entomology* 45(3) May:414–420.

Pruthi, H.S., and H.N. Batra. 1938. A preliminary annotated list of fruit pests of the North-West Frontier Province. *Miscellaneous Bulletin of the Imperial Council of Agricultural Research in India* 19:10–12.

Purcell, A.H. 1982. Insect vector relationships with procaryotic plant pathogens. *Annual Review of Phytopathology* 20(1):397–417.

Purcell, A.H., and L.R. Nault. 1991. Interactions among plant pathogenic prokaryotes, plants, and insect vectors. In *Microbial Mediation of Plant-Herbivore Interactions*. eds. P.

Barbosa, V.A. Krischik and C.G. Jones. pp. 383–405 New York: John Wiley and Sons Inc.

Purcell, A.H., and K.G. Suslow. 1987. Pathogenicity and effects on transmission of a mycoplasmalike organism of a transovarially infective bacterium on the leafhopper *Euscelidius variegatus* (Homoptera: Cicadellidae). *Journal of Invertebrate Pathology* 50(3):285-290.

Reddy, K.N., M. Singh, and A.K. Alva. 1992. Sorption and leaching of bromacil and simazine in Florida flatwoods soils. *Bulletin of Environmental Contamination and Toxicology* 48(5):662–670.

Reinking, O.A. 1919. Diseases of economic plants in Southern China. *Philippine Agriculturist* 8:109–135.

RGG (Research Grants and Contracts Group). 2009. What is a contract? May 01, 2009. Melbourne, Australia: The University of Melbourne. Available online at http://www.research.unimelb.edu.au/rgc/contracts/definition. Accessed August 6, 2009.

Rogers, M.E., and M.M. Dewdney. 2010. 2010 Florida citrus pest management Guide: Pesticide resistance and resistance management. Florida Citrus Pest Management Guide. November 2009. ENY-624. Gainesville: University of Florida/IFAS. Available online at http://edis.ifas.ufl.edu/cg026. Accessed December 16, 2009.

Rogers, M.E., and P.A. Stansly. 2006. Biology and management of the Asian citrus psyllid, *Diaphorina citri* Kuwayama, in Florida citrus. Featured Creatures. Revised: March 21 2007. ENY-739. Gainesville: University of Florida. Available online at http://edis.ifas.ufl.edu/IN668. Accessed July 25, 2009.

Rogers, M.E., P.A. Stansly, and L.L. Stelinski. 2010. 2010 Florida citrus pest management guide: Asian citrus psyllid and citrus leafminer. Florida Citrus Pest Management Guide. November 2009. ENY-734. Gainesville: University of Florida/IFAS. Available online at http://edis.ifas.ufl.edu/IN686. Accessed December 16, 2009.

Roistacher, C.N. 1991. Graft-Transmissible Diseases of Citrus: Handbook for Detection and Diagnosis. Rome: Food and Agriculture Organization of the United Nations - International Organization of Citrus Virologists.

———. 1996. The economics of living with citrus Diseases: Huanglongbing (greening) in Thailand. In Proceedings of the 13th Conference of the International Organization of Citrus Virologists, eds. P. Moreno, J.V. da Graça and L.W. Timmer, pp. 279–285, November 16–23, 1995, at Fouzhou, China. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/ proceedingsXIII/13th279_285.pdf. Accessed November 10, 2009http://www.ivia.es/iocv/archivos/proceedingsXIII/13th279_285.pdf.

Román, M.P., M. Cambra, J. Juárez, P. Moreno, N. Duran-Vila, F.A.O. Tanaka, E. Alves, E.W. Kitajima, P.T. Yamamoto, R.B. Bassanezi, D.C. Teixeira, W.C. Jesus Jr, A.J. Ayres, N. Gimenes-Fernandes, F. Rabenstein, L.F. Girotto, and J.M. Bové. 2004. Sudden death of citrus in Brazil: A graft-transmissible bud union disease. *Plant Disease* 88(5):453–467.

Roose, M.L., and T.J. Close. 2008. Plant genetics and genomics: Crops and models. In *Genomics of Tropical Crop Plants*, *Plant genetics and genomics: Crops and models*. Vol. 1. eds. P.H. Moore, R. Ming and D.P. Delmer. pp. 187–202 New York: Springer.

Rothschild, E.R., R.J. Manser, and M.P. Anderson. 1982. Investigation of aldicarb in ground water in selected areas of the Central Sand Plain of Wisconsin. *Ground Water* 20(4):437–445.

Rouseff, R.L., E.O. Onagbola, J.M. Smoot, and L.L. Stelinski. 2008. Sulfur volatiles in guava (*Psidium guajava* L.) leaves: Possible defense mechanism. *Journal of Agricultural and Food Chemistry* 56(19):8905–8910.

Roush, R.T. 1989. Designing resistance management programs: How can you choose? *Pesticide Science* 26(4):423–441.

Saglio, P., D. Laflèche, C. Bonissol, and J.M. Bové. 1971. Isolement, culture et observation au microscope électronique des structures de type mycoplasme associées à la maladie du stubborn des agrumes et leur comparaison avec des structures observées dans le cas de la maladie du greening des agrumes. *Physiologie Végétale* 9:569–582.

Salibe, A.A., and R.E. Cortez. 1968. Leaf mottling, a serious virus disease of citrus in the Philippines. In Proceedings of the 4th Conference of International Organization of Citrus Virologists, pp. 131–136, October 2–12, 1966, at Catania, Italy. Gainesville: University of Florida Press. Available online at http://www.ivia.es/iocv/archivos/proceedings/4/4th131_136.pdf. Accessed August 6, 2009.

Sauvion, N., C. Nardon, G. Febvay, A.M.R. Gatehouse, and Y. Rahbé. 2004. Binding of the insecticidal lectin Concanavalin A in pea aphid, *Acyrthosiphon pisum* (Harris) and induced effects on the structure of midgut epithelial cells. *Journal of Insect Physiology* 50(12):1137-1150.

Schetelig, M.F., C. Caceres, A. Zacharopoulou, G. Franz, and E.A. Wimmer. 2009. Conditional embryonic lethality to improve the sterile insect technique in *Ceratitis capitata* (Diptera: Tephritidae). *BMC Biology* 7(4):10.1186/1741-7007-7-4.

Schreiber, K., W. Ckurshumova, J. Peek, and D. Desveaux. 2008. A high-throughput chemical screen for resistance to *Pseudomonas syringae* in Arabidopsis. *Plant Journal* 54(3):522–531.

Schubert, T.S., S.A. Rizvi, X. Sun, T.R. Gottwald, J.H. Graham, and W.N. Dixon. 2001. Meeting the challenge of eradicating citrus canker in Florida—again. *Plant Disease* 85(4):340–356.

Schwarz, R.E., and S.P. von Vuuren. 1971. Decreasing fruit greening of sweet orange by trunk injections of tetracycline. *Plant Disease Reporter* 55:747–750.

Schwarz, R.E., L.C. Knorr, and M. Prommintara. 1973. Presence of citrus greening and its psylla vector in Thailand. *FAO Plant Protection Bulletin* 21:132–138.

Schwarz, R.E., J.N. Moll, and S.P. van Vuuren. 1974. Control of citrus greening of citrus psylla vector by trunk injections of tetracycline and insecticides. In Proceedings of the 6th Conference of International Organization of Citrus Virologists, eds. L.G. Weathers and M. Cohen, pp. 26–29, August 21–28, at Swaziland. Riverside, CA: IOCV.

Scorza, R., J.-M. Hily, A. Callahan, T. Malinowski, M. Cambra, N. Capote, I. Zagrai, V. Damsteegt, P. Briard, and M. Ravelonandro. 2007. Deregulation of plum pox resistant transgenic plum "honeysweet". *Acta Horticulturae (ISHS)* 738:669–673.

Sechler, A., E.L. Schuenzel, P. Cooke, S. Donnua, N. Thaveechai, E. Postnikova, A.L. Stone, W.L. Schneider, V.D. Damsteegt, and N.W. Schaad. 2009. Cultivation of '*Candidatus* Liberibacter asiaticus', '*Ca*. L. africanus', and '*Ca*. L. americanus' associated with huanglongbing. *Phytopathology* 99(5):480–486.

Secor, G.A., and V.V. Rivera-Varas. 2004. Emerging diseases of cultivated potato and their impact on Latin America. *Revista Latinoamericana de la Papa* 1(Suppl.):1–8.

Seif, A.A., and R.J. Hillocks. 1997. Chemical control of *Phaeoramularia* fruit and leaf spot of citrus in Kenya. *Crop Protection* 16(2):141–145.

———. 1998. Some factors affecting infection of citrus by *Phaeoramularia angolensis*. *Journal of Phytopathology* 146(8-9):385–391.

Semancik, J.S. 2003. Considerations for the introduction of viroids for economic advantage. In *Viroids*. eds. A. Hadidi, R. Flores, J. Randles and J.S. Semancik. pp. 357-362. Canberra, Australia: CSIRO Publishing.

Shafee, S.M., S.M. Alam, and M.M. Agarwal. 1975. Taxonomic survey of encyrtid parasites (Hymenoptera: Encyrtidae) in India. In *Aligarh Muslim University Publication (Zoological Series) on Indian Insect Types*. ed. S.M. Alam. pp. 91–93 Aligarh, India.

Shelton, A.M., and F.R. Badenes-Perez. 2006. Concepts and applications of trap cropping in pest management. *Annual Review of Entomology* 51:285–308.

Singh, S., and M.V. Rajam. 2009. Citrus biotechnology: Achievements, limitations and future directions. *Physiology and Molecular Biology of Plants* 15(1):3–22.

Skelley, L.H., and M.A. Hoy. 2004. A synchronous rearing method for the Asian citrus psyllid and its parasitoids in quarantine. *Biological Control* 29(1):14–23.

Soehnlein, O. 2009. Direct and alternative antimicrobial mechanisms of neutrophil-derived granule proteins. *Journal of Molecular Medicine* 87(12):1157-1164.

Soost, R.K., and M.L. Roose. 1996. Citrus. In *Fruit Breeding: Trees and Tropical Fruit*, Vol. 1. eds. J. Janick and J.N. Moore. pp. 257–323 New York: Wiley.

Spreen, T., M.G. Brown, and C. Jauregui. 2008. Production and price effects of new diseases and other challenges confronting the processed orange industry. Paper presented at the 2008 Southern Agricultural Economics Association Annual Meeting, February 2–6, in Dallas, TX. Southern Agricultural Economics Association. Available online at http://ideas.repec.org/p/ags/saeaed/6715.html. Accessed May 4, 2009.

Srivastava, A.C., S. Ganesan, I.O. Ismail, and B.G. Ayre. 2009. Effective carbon partitioning driven by exotic phloem-specific regulatory elements fused to the *Arabidopsis thaliana* AtSUC2 sucrose-proton symporter gene. *BMC Plant Biology* 9(7):10.1186/1471-2229-9-7.

Stahel, G. 1915. *Marasmius perniciosus* nov. spec. The cause of krulloten disease of cocoa in Suriname. Bulletin No. 33. Paramaribo: Department van der Landbounin Suriname.

Stanisçuaski, F., C.T. Ferreira-DaSilva, F. Mulinari, M. Pires-Alves, and C.R. Carlini. 2005. Insecticidal effects of canatoxin on the cotton stainer bug *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae). *Toxicon* 45(6):753-760.

Stapleton, J.J., and C.G. Summers. 2002. Reflective mulches for management of aphids and aphid-borne virus diseases in late-season cantaloupe (*Cucumis melo* L. var. *cantalupensis*). *Crop Protection* 21(10):891-898.

Storer, N.P., J.W. Van Duyn, and G.G. Kennedy. 2001. Life history traits of *Helicoverpa zea* (Lepidoptera: Noctuidae) on non-Bt and Bt transgenic corn hybrids in eastern North Carolina. *Journal of Economic Entomology* 94(5):1268–1279.

Stover, E.W., T.R. Gottwald, D.G. Hall, M.K. Aradhya, and F.T. Zee. 2008. Guava SSR analysis: Diversity assessment and similarity to accessions associated with reducing citrus greening in Vietnam. Meeting Abstract. *HortScience* 43(4) July 21–24, 2008:1119.

Su, H.J., and S.C. Chang. 1974. The responses of likubin pathogen to antibiotics and heat therapy. In Proceedings of the 6th Conference of International Organization of Citrus Virologists, eds. L.G. Weathers and M. Cohen, pp. 27–34, August 21–28, at Swaziland. Riverside, CA: IOCV.

Subandiyah, S., T. Iwanami, S. Tsuyumu, and H. Ieki. 2000. Comparison of 16S rDNA and 16S/23S intergenic region sequences among citrus greening organisms in Asia. *Plant Disease* 84(1):15–18.

Supriyanto, A., and A.M. Whittle. 1991. Citrus rehabilitation in Indonesia. In Proceedings of the 11th Conference of the International Organization of Citrus Virologists, eds. R.H. Brlansky, R.F. Lee and L.W. Timmer, pp. 409–413, November 6–10, 1989, at Orlando, FL. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsXI/11th409_413.pdf. Accessed September 12, 2009.

Supriyanto, A., and A.M. Whittle. 1991. Citrus rehabilitation in Indonesia. In Proceedings of the 11th Conference of the International Organization of Citrus Virologists, eds. R.H. Brlansky, R.F. Lee and L.W. Timmer, pp. 409–413, November 6–10, 1989, at Orlando, FL. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsXI/11th409_413.pdf. Accessed September 12, 2009.

Talon, M., and F.G. Gmitter, Jr. 2008. Citrus genomics. *International Journal of Plant Genomics* 2008(Article ID 528361):10.1155/2008/528361.

Tatineni, S., U.S. Sagaram, S. Gowda, C.J. Robertson, W.O. Dawson, T. Iwanami, and N. Wang. 2008. *In planta* distribution of '*Candidatus* Liberibacter asiaticus' as revealed by polymerase chain reaction (PCR) and real-time PCR. *Phytopathology* 98(5):592–599.

Teixeira, D.C., J. Ayres, E.W. Kitajima, L. Danet, S. Jagoueix-Eveillard, C. Saillard, and J.M. Bové. 2005a. 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(1):107.

Teixeira, D.C., C. Saillard, S. Eveillard, J.L. Danet, P.I. da Costa, A.J. Ayres, and J. Bové. 2005b. '*Candidatus* Liberibacter americanus', associated with citrus huanglongbing (greening disease) in São Paulo State, Brazil. *International Journal of Systematic and Evolutionary Microbiology* 55(5):1857–1862.

Teixeira, D.C., J.L. Danet, S. Eveillard, E.C. Martins, W.C. De Jesus Jr, P.T. Yamamoto, S.A. Lopes, R.B. Bassanezi, A.J. Ayres, C. Saillard, and J.M. Bové. 2005c. Citrus huanglongbing in São Paulo State, Brazil: PCR detection of the '*Candidatus*' Liberibacter species associated with the disease. *Molecular and Cellular Probes* 19(3):173–179.

Teixeira, D.C., C. Saillard, S. Eveillard, J.L. Danet, A.J. Ayres, and J.M. Bové. 2005d. A new Liberibacter species, *Candidatus* Liberibacter americanus sp. nov., is associated with citrus huanglongbing (greening disease) in São Paulo State, Brazil. In Insect-Transmitted Procaryotes: Proceedings of the 16th Conference of the International Organization of Citrus Virologists, eds. M.E. Hilf, N. Duran-Vila and M.A. Rocha-Peña, pp. 325–340, November 7–12, 2004, at Monterrey, Mexico. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/ProceedingsXV/proceedings%20XVI%20conference/325-340%20IOCV%2016.pdf. Accessed February 25, 2009.

Teixeira, D.C., S.A. Lopes, P.T. Yamamoto, S. Eveillard, E.C. Martins, W.C. de Jesus Junior, R.B. Bassanezi, A.J. Ayres, J.L. Danet, C. Saillard, and J.M. Bové. 2005e. PCR detection of the two Liberibacter species associated with citrus huanglongbing in São Paulo State, Brazil. In Proceedings of the 16th Conference of the International Organization of Citrus

Virologists, eds. M.E. Hilf, N. Duran-Vila and M.A. Rocha-Peña, pp. 432–438, November 7–12, 2004, at Monterrey, Mexico. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/ProceedingsXV/proceedings%20XVI%20conference/432-438%20IOCV%2016.pdf. Accessed February 17, 2009.

Teixeira, D.C., S. Eveillard, P. Sirand-Pugnet, A. Wulff, C. Saillard, A.J. Ayres, and J.M. Bové. 2008a. The tufB-secE-nusG-rplKAJL-rpoB gene cluster of the Liberibacters: Sequence comparisons, phylogeny and speciation. *International Journal of Systematic and Evolutionary Microbiology* 58(6):1414–1421.

Teixeira, D.C., C. Saillard, C. Couture, E.C. Martins, N.A. Wulff, S. Eveillard-Jagoueix, P.T. Yamamoto, A.J. Ayres, and J.M. Bové. 2008b. Distribution and quantification of *Candidatus* Liberibacter americanus, agent of huanglongbing disease of citrus in São Paulo State, Brasil, in leaves of an affected sweet orange tree as determined by PCR. *Molecular and Cellular Probes* 22(3):139-150.

Teixeira, D.C., N.A. Wulff, E.C. Martins, E.W. Kitajima, R. Bassanezi, A.J. Ayres, S. Eveillard, C. Saillard, and J.M. Bové. 2008c. A Phytoplasma closely related to the pigeon pea witches'-broom Phytoplasma (16Sr IX) is associated with citrus huanglongbing symptoms in the State of São Paulo, Brazil. *Phytopathology* 98(9):977–984.

Thomas, M.C., J.B. Heppner, R.E. Woodruff, H.V. Weems, G.J. Steck, and T.R. Fasulo. 2007. Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Insecta: Diptera: Tephritidae). EENY-214. Gainesville: Institute of Food and Agricultural Sciences, University of Florida. Available online at http://edis.ifas.ufl.edu/IN371. Accessed September 23, 2009.

Tilling, A.K., G.J. O'Leary, J.G. Ferwerda, S.D. Jones, G.J. Fitzgerald, D. Rodriguez, and R. Belford. 2007. Remote sensing of nitrogen and water stress in wheat. *Field Crops Research* 104(1-3):77–85.

Timmer, L.W., and A. Bhatia. 2003. Diseases of unknown etiology. In *Plant Microbe Interactions*. Vol. Vol. 6. eds. G. Stacey and N.T. Keen. pp. 309–329 St. Paul, MN: APS Press.

Timmer, L.W., and G.E. Brown. 2000. Biology and control of anthracnose diseases of citrus. In *Colletotrichum: Host Specificity, Pathology and Host-Pathogen Interaction*. eds. D. Prusky, S. Freeman and M. Dickman. St. Paul, MN: APS Press.

Timmer, L.W., J.P. Agostini, S.E. Zitko, and M. Zulfiqar. 1994. Postbloom fruit drop, an increasingly prevalent disease of citrus in the Americas. *Plant Disease* 78(4):329–334.

Timmer, L.W., S.M. Garnsey, and J.H. Graham, eds. 2000. Compendium of Citrus Diseases. 2nd ed. St. Paul, MD: APS Press.

Timmer, L.W., S.M. Garnsey, and P. Broadbent. 2003. Diseases of citrus. In *Diseases of Tropical Fruit Crops*. ed. R.C. Ploetz. pp. 163–195 Wallingford, Oxon, UK: CABI Publishers.

Timmer, L.W., S.N. Mondal, N.A.R. Peres, and A. Bhatia. 2004. Fungal diseases of fruit and foliage of citrus trees. In *Diseases of Fruits and Vegetables: Diagnosis and Management*. Vol. 1. ed. S.A.M.H. Naqvi. pp. 191–227. The Netherlands: Kluwer Academic Publishers.

Timmer, L.W., and L.W. Duncan, eds. 1999. Citrus Health Management, Plant Health Management. St. Paul, MN: APS Press.

Tomimura, K., S.I. Miyata, N. Furuya, K. Kubota, M. Okuda, S. Subandiyah, T.H. Hung, H.J. Su, and T. Iwanami. 2009. Evaluation of genetic diversity among '*Candidatus*

Liberibacter asiaticus' isolates collected in Southeast Asia. *Phytopathology* 99(9):1062–1069.

Trivedi, P., U.S. Sagaram, J.S. Kim, R.H. Brlansky, M.E. Rogers, L.L. Stelinski, C. Oswalt, and N. Wang. 2009. Quantification of viable *Candidatus* Liberibacter asiaticus in hosts using quantitative PCR with the aid of ethidium monoazide (EMA). *European Journal of Plant Pathology* 124(4) Aug:553–563.

Tsai, J.H., and Y.H. Liu. 2000. Biology of *Diaphorina citri* (Homoptera: Psyllidae) on four host plants. *Journal of Economic Entomology* 93(6):1721–1725.

Tsai, J.H., J.J. Wang, and Y.H. Liu. 2002. Seasonal abundance of the Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae) in Southern Florida. *Florida Entomologist* 85(3):446–451.

Turgeon, R., and S. Wolf. 2009. Phloem transport: Cellular pathways and molecular trafficking. *Annual Review of Plant Biology* 60(1):207–221.

Tyler, H.L., L.F.W. Roesch, S. Gowda, W.O. Dawson, and E.W. Triplett. 2009. Confirmation of the sequence of '*Candidatus* Liberibacter asiaticus' and assessment of microbial diversity in huanglongbing-infected citrus phloem using a metagenomic approach. *Molecular Plant-Microbe Interactions* 22(12):1624–1634.

U.S. Census Bureau. 2009. Population estimates program (T1). from U.S. Census Bureau, Available online at http://factfinder.census.gov/servlet/DTTable?_bm=y&-geo_id=04000US12&-ds_name=PEP_2008_EST&-mt_name=PEP_2008_EST_G2008_T001. Accessed August 21, 2009.

UNCTAD (United Nations Conference on Trade and Development) Info Comm. 2009. Origin and history. January 25, 2005. United Nations Conference on Trade and Development. Available online at http://www.unctad.org/infocomm/anglais/orange/characteristics.htm#hist. Accessed August 4, 2009.

USDA-APHIS (U.S. Department of Agriculture - Animal Plant Health Inspection Service). 2006. Exotic fruit fly strategic plan FY 2006-2010. June 19. Riverdale, MD: U.S. Department of Agriculture, Animal Plant Health Inspection Service. Available online at http://www.aphis.usda.gov/plant_health/plant_pest_info/fruit_flies/downloads/strategicplan06-19-06.pdf. Accessed July 6, 2009.

———. 2009. Area wide control of Asian citrus psyllid (*Diaphorina citri*): Technical working group report. February 09. Final ed. Washington, DC: Animal and Plant Health Inspection Service. Available online at http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/twg/Psyllid%20Area%20Wide%20Control2.09.09.pdf. Accessed January 30, 2010.

USDA-ARS (U.S. Department of Agriculture - Agricultural Research Service). 2005. Specific cooperative agreements with the Agricultural Research Service: Guidelines for partner institutions. Washington, DC: U.S. Department of Agriculture-Agricultural Research Service. Available online at http://www.ars.usda.gov/is/np/SpecCoopAgreements/SpecCoopAgreementsintro.htm. Accessed January 30, 2010.

USDA-CSREES (U.S. Department of Agriculture - Cooperative State Research Education and Extension Service). 2009. Funding mechanisms. January 15. Version 1. Washington, DC: U.S. Department of Agriculture - Cooperative State Research Education and Extension Service. Available online at http://www.csrees.usda.gov/about/pdfs/funding_mechanisms.pdf. Accessed February 27, 2009.

USDA-FAS (U.S. Department of Agriculture - Foreign Agricultural Service). 2008. Brazil citrus annual 2008. December 19. BR8021. Washington, DC: U.S. Department of Agriculture - Foreign Agricultural Service. Available online at http://www.fas.usda.gov/gainfiles/200812/146306943.pdf. Accessed February 27, 2009.

USDA-NASS (U.S. Department of Agriculture - National Agricultural Statistical Service). 2008. Citrus fruits 2008 summary. September. Fr Nt 3-1 (08). Washington, DC: U.S. Department of Agriculture - National Agricultural Statistical Service. Available online at http://usda.mannlib.cornell.edu/usda/nass/CitrFrui//2000s/2008/CitrFrui-09-25-2008.pdf. Accessed January 30, 2010.

USDA-NASS (U.S. Department of Agriculture - National Agricultural Statistical Service - Florida Field Office). 2007. 2004-05 Florida Citrus Summary. January. Orlando, FL: U.S. Department of Agriculture - NASS/Florida Agricultural Statistical Service Available online at http://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/cs/2005-06/CS0506all.pdf. Accessed February 27, 2009.

———. 2009. Citrus summary 2007-08. March. Orlando, FL: U.S. Department of Agriculture - NASS/Florida Agricultural Statistical Service Available online at http://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/cs/2007-08/cs0708all.pdf. Accessed February 27, 2009.

USDA-NIFA (U.S. Department of Agriculture - National Institute of Food and Agriculture). 2010a. Agriculture and food research initiative competitive grants program. November 5, 2009. Washington, DC: U.S. Department of Agriculture - National Institute of Food and Agriculture. Available online at http://www.csrees.usda.gov/fo/agriculturalandfoodresearchinitiativeafri.cfm. Accessed January 30, 2010.

———. 2010b. Special research grants program: Pest management alternatives program. January 7, 2010. Washington, DC: U.S. Department of Agriculture - National Institute of Food and Agriculture. Available online at http://www.csrees.usda.gov/fo/pestmanagementalternativessrgp.cfm. Accessed January 30, 2010.

———. 2010c. Integrated Pest Management. December 17, 2009. Washington, DC: U.S. Department of Agriculture - National Institute of Food and Agriculture. Available online at http://www.csrees.usda.gov/integratedpestmanagement.cfm. Accessed January 30, 2010.

———. 2010d. Interregional research project #4 minor crop pest management program (IR-4). October 19, 2009. Washington, DC: U.S. Department of Agriculture - National Institute of Food and Agriculture. Available online at http://www.csrees.usda.gov/fo/ir4minorcroppestmanagement.cfm. Accessed January 30, 2010.

———. 2010e. Specialty crop research initiative. November 12, 2009. Washington, DC: U.S. Department of Agriculture - National Institute of Food and Agriculture. Available online at http://www.nifa.usda.gov/fo/specialtycropresearchinitiative.cfm. Accessed January 30, 2010.

———. 2010f. Special research grants program: *Citrus tristeza* research (CTV) program. December 12, 2006. Washington, DC: U.S. Department of Agriculture - National Institute of Food and Agriculture. Available online at http://www.nifa.usda.gov/fo/fundview.cfm?fonum=1079. Accessed January 30, 2010.

Vaccaro, N.C. 1994. Comportamiento y diffusion de la “chicharrita de los citrus” *Diaphorina citri* en la zona citrica de Entre Rios. *Información Citricota* CITRUS vol.2:INTA – E.E.A.

Van Den Berg, M.A., S.P.v. Vuuren, and V.A. Deacon. 1992. Studies on greening disease transmission by the citrus psylla, *Trioza erytreae* (Hemiptera: Triozidae). *Israel Journal of Entomology* 25-26(51-56).

Van der Merwe, A.J., and F.G. Andersen. 1937. Chromium and manganese toxicity. Is it important in Transvaal citrus greening? *Farming South Africa* 12:439–440.

Vialatte, A., M. Plantegenest, J.-C. Simon, and C.-A. Dedryver. 2007. Farm-scale assessment of movement patterns and colonization dynamics of the grain aphid in arable crops and hedgerows. *Agricultural and Forest Entomology* 9(4):337-346.

Vikram, A., B. Prithiviraj, H. Hamzehzarghani, and A.C. Kushalappa. 2004. Volatile metabolite profiling to discriminate diseases of McIntosh apple inoculated with fungal pathogens. *Journal of the Science of Food and Agriculture* 84(11):1333–1340.

Villalobos, W., D. Hollis, C. Godoy, and C. Rivera. 2005. First report of *Diaphorina citri* (Hemiptera: Psyllidae) in Costa Rica. *Insecta Mundi* 19(3):191–192.

Villechanoux, S., M. Garnier, J. Renaudin, and J.M. Bové. 1992. Detection of several strains of the bacterium-like organism of citrus greening disease by DNA probes. *Current Microbiology* 24(2):89–95.

Villechanoux, S., M. Garnier, F. Laigret, J. Renaudin, and J.M. Bové. 1993. The genome of the non-cultured, bacterial-like organism associated with citrus greening disease contains thenusG-rplKAJL-rpoBC gene cluster and the gene for a bacteriophage type DNA polymerase. *Current Microbiology* 26(3):161–166.

Wang, C.X., N. Hong, G.P. Wang, B. Jiang, and X.D. Fan. 2009. Effects of *Citrus tristeza* virus on the growth of in vitro-cultured citrus. *Journal of Plant Pathology* 91(2):357–363.

Wang, Z., Y. Yin, H. Hu, Q. Yuan, G. Peng, and Y. Xia. 2006. Development and application of molecular-based diagnosis for ‘*Candidatus* Liberibacter asiaticus’, the causal pathogen of citrus huanglongbing. *Plant Pathology* 55(5):630–638.

Webber, H.J., W. Reuther, and H.W. Lawton. 1967. History and development of the citrus industry. In *The Citrus Industry*, Vol. 1. eds. W. Reuther, H.J. Webber and L.D. Batchelor. p. 374. Revised edition. Riverside: University of California Board of Regents.

Weems, H.V., J.B. Heppner, J.L. Nation, and T.R. Fasulo. 2008. Oriental fruit fly, *Bactrocera (= Dacus) dorsalis* (Hendel) (Insecta: Diptera: Tephritidae). July. EENY-083. Gainesville: Institute of Food and Agricultural Sciences, University of Florida. Available online at http://edis.ifas.ufl.edu/IN240. Accessed April 2, 2009.

Weisburg, W.G., S.M. Barns, D.A. Pelletier, and D.J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* 173(2):697–703.

Wenninger, E.J., and D.G. Hall. 2007. Daily timing of mating and age at reproductive maturity in *Diaphorina citri* (Hemiptera: Psyllidae). *Florida Entomologist* 90(4):715–722.

———. 2008. Importance of multiple mating to female reproductive output in *Diaphorina citri*. *Physiological Entomology* 33(4):316–321.

Wenninger, E.J., L.L. Stelinski, and D.G. Hall. 2008. Behavioral evidence for a female-produced sex attractant in *Diaphorina citri*. *Entomologia Experimentalis et Applicata* 128(3):450–459.

Wenninger, E.J., D.G. Hall, and R.W. Mankin. 2009a. Vibrational communication between the sexes in *Diaphorina citri* (Hemiptera: Psyllidae). *Annals of the Entomological Society of America* 102(3):547–555.

Wenninger, E.J., L.L. Stelinski, and D.G. Hall. 2009b. Roles of olfactory cues, visual cues, and mating status in orientation of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) to four different host plants. *Environmental Entomology* 38(1):225–234.

Werner, B.J., T.M. Mowry, N.A. Bosque-Pérez, H. Ding, and S.D. Eigenbrode. 2009. Changes in green peach aphid responses to potato leafroll virus—induced volatiles emitted during disease progression. *Environmental Entomology* 38(5):1429-1438.

Whitlock, V.H., and R.-K. Chippindall. 1993. Greening disease in South Africa: A multi-pathogen syndrome? In Proceedings of the 12th Conference of the International Organization of Citrus Virologists, eds. P. Moreno, J.V. da Graça and L.W. Timmer, pp. 233–243, November 23–27, 1992, at New Delhi, India. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsXII/12th233_243.pdf. Accessed September 1, 2009.

Woodruff, R.E. 1985. Citrus weevils in Florida and the West Indies: Preliminary report on systematics, biology, and distribution (Coleoptera: Curculionidae). *Florida Entomologist* 68(3):370–379.

Wulff, N.A., S. Eveillard, X. Foissac, A.J. Ayres, and J.M. Bové. 2009. Ribosomal RNA operons and genome size of *'Candidatus* Liberibacter americanus', a bacterium associated with citrus huanglongbing in Brazil. *International Journal of Systematic and Evolutionary Microbiology* 59(8):1984–1991.

Xu, C.F., K.B. Li, C. Ke, and J.Z. Liao. 1985. On the transmission of citrus yellow shoot by psylla and observation with electron microscopy. *Acta Physiologiae Sinica* 15(4):241–245.

Xu, C.F., Y.H. Xia, K.B. Li, and C. Ke. 1988. Further study of the transmission of citrus huanglongbing by a psyllid, *Diaphorina citri* Kuwayama. In Proceedings of the 10th Conference of International Organization of Citrus Virologists, eds. L.W. Timmer, S.M. Garnsey and L. Navarro, pp. 243–248, November 17–21, 1986, at Valencia, Spain. Riverside, CA: IOCV. Available online at http://www.ivia.es/iocv/archivos/proceedingsX/10th243_248.pdf. Accessed March 7, 2009.

Zeiri, L. 2007. SERS of plant material. *Journal of Raman Spectroscopy* 38(7):950-955.

Zhang, A., and J.S. Hartung. 2005. Phenylacetaldehyde O-methyloxime: A volatile compound produced by grapefruit leaves infected with the citrus canker pathogen, *Xanthomonas axonopodis* pv. *citri*. *Journal of Agricultural and Food Chemistry* 53(13):5134–5137.

Zhang, B., X. Pan, G.P. Cobb, and T.A. Anderson. 2006. Plant microRNA: A small regulatory molecule with big impact. *Developmental Biology* 289(1):3-16.

Zhang, M., Y. Duan, L. Zhou, W.W. Turechek, E. Stover, and C.A. Powell. 2010. Screening molecules for control of citrus huanglongbing using an optimized regeneration system for '*Candidatus* Liberibacter asiaticus'-infected periwinkle (*Catharanthus roseus*) cuttings. *Phytopathology* 100(3):239–245.

Zhao, W., S. Sankaran, A.M. Ibanez, A.M. Dandekar, and C.E. Davis. 2009. Two-dimensional wavelet analysis based classification of gas chromatogram differential mobility spectrometry signals. *Analytica Chimica Acta* 647(1) Aug 4:46–53.

Zhou, L.J., D.W. Gabriel, Y.P. Duan, S.E. Halbert, and W.N. Dixon. 2007. First report of dodder transmission of huanglongbing from naturally infected *Murraya paniculata* to citrus. Notes. *Plant Disease* 91(2):227–227.

Zhou, L., Y. Duan, D. Gabriel, and T.R. Gottwald. 2008a. Seed transmission of *Candidatus* Liberibacter asiaticus in periwinkle and dodder resulted in low bacterial titer and very mild disease in periwinkle. Abstract. *Phytopathology* 98(6):S181.

Zhou, X., M.M. Wheeler, F.M. Oi, and M.E. Scharf. 2008b. RNA interference in the termite *Reticulitermes flavipes* through ingestion of double-stranded RNA. *Insect Biochemistry and Molecular Biology* 38(8):805–815.

Appendixes

Appendix A

Committee Statement of Task

An NRC committee of approximately 12–15 experts will be appointed to develop a strategic plan to use science and technology to respond to citrus greening and other diseases that threaten the Florida citrus industry. The plan will define key objectives for finding and implementing a solution to citrus greening, including major basic and applied research themes, technology development and distribution strategies, approaches for controlling the spread of the disease and sustaining the industry, and options for strengthening the ability of the industry to respond in a comprehensive way to citrus greening and future disease threats.

In developing the plan, the committee will examine:

- The current citrus disease situation in Florida and the status of public and private efforts to address citrus greening and other diseases, including lessons learned

- The capacity of the industry to mobilize a scientifically-based response to current disease threats and to translate scientific advances into products and services for the protection of Florida citrus industry in the short and long term; and

- The relationship of the industry to public, academic, and private research, and to regulatory and funding organizations at the state and federal level, with respect to controlling citrus greening and developing a comprehensive solution to citrus greening and other diseases.

In developing the plan, the committee will take note of ongoing research and technology development and commercialization activities underway across the United States, in the federal research laboratories, and in foreign research institutions, and identify opportunities where collaboration might be timely or cost-effective or where research activities could be combined (control or regulatory activities, for example) to achieve synergy. The committee's report will explore alternate models for organizing research, development, and implementation of scientific and technological tools for fighting the current (and future) disease problems, relative to the industry's strengths and sphere of influence.

Recognizing the urgency of the current problem of citrus greening, the committee's plan will highlight activities that could improve the ability to respond to citrus greening by filling critical gaps in knowledge or improving the capacity to implement innovations or control the disease. The report will estimate of the cost, difficulty, and timeframe for completing key objectives of the strategic plan.

Appendix B

Committee Biographies

George E. Bruening, *Chair***,** is a Professor Emeritus at University of California, Davis**.** Dr. Bruening's research interests are in the area of replication mechanisms of plant viruses and how these mechanisms may be interdicted by plant genes, subviral agents, or engineered genes. He specializes in plant resistance against viruses and bacteria and biotechnology in plant pathology and agriculture. He has held several editorial positions including: Associate Editor and Editor of *Virology* and founding Editor-in-Chief of *Molecular Plant-Microbe Interactions* journals. Dr. Bruening is a member of the California Department of Food and Agriculture Pierce's Disease Advisory Task Force, 2007-present. He was the director of the Center for Engineering Plants for Resistance Against Pathogens, National Science Foundation Science and Technology Center at the University of California, Davis, from 1991-2002. He currently serves as the director of the University of California Pierce's Disease Grants Program, a position he has held since 2006. He teaches general undergraduate courses in agricultural biotechnology and graduate courses in plant virology and general virology. Dr. Bruening has been widely recognized through honors such as: the NSF Postdoctoral Fellowship, 1965–1966; the John Simon Guggenheim Memorial Foundation fellowship, 1974–1975; and the Award of Distinction, College of Agricultural and Environmental Sciences, University of California at Davis, 2008. He was elected as a National Academy of Science member in 1992. He served as the Chair of the Parent Committee on the Review of Citrus Greening Proposals in 2008. Dr. Bruening received his Ph.D. in Biochemistry from the University of Wisconsin, Madison.

Joseph-Marie Bové has been an Emeritus Professor of microbiology at the University of Bordeaux since 1999. Prior to this, Dr. Bové was President of the National Institute for Agricultural Research Center of Bordeaux from 1984 to 1994. He served from 1981 to 1992 and 2004 to 2007 as part-time consultant for the Food and Agriculture Organization (FAO) to survey for citrus diseases in the Near East and the Middle East. Results of the surveys have been published by FAO in the form of a book (1995). During these surveys, he discovered huanglongbing (HLB) in Saudi Arabia, Yemen, Somalia, Laos, Myanmar, Cambodia, Vietnam and Bhutan. He is a member of the French Academy of Agriculture, the French Academy of Science, the Brazilian Academy of Science, the American Academy of Microbiology and is an elected fellow of the American Phytopathological Society. He is also a member of the International Organization of Citrus Virologists; he served as its president from 1969 to 1972. He served as the president of the International Organization for Mycoplasmology, from 1992 to 1994. Since 2004, when HLB was identified in Brazil, this destructive disease of citrus has become Bové's major occupation in Brazil and other affected countries. Dr. Bové has over 250 publications in international refereed journals. He holds a Ph.D. from the University of Paris, France.

Paul Citron retired from Medtronic Inc. in 2003 as Vice President of Technology Policy and Academic Relations. In that position he was responsible for identifying and addressing public policy matters that affect medical technology innovation and for working with leading biomedical engineering institutions. He is currently an adjunct faculty member at University of California (UC), San Diego and UC Riverside, Department of Bioengineering. Mr. Citron was elected to the National Academy of Engineering in 2003; he was elected as a Founding Fellow of the American Institute of Medical and Biological Engineering in 1993; he received the American College of Cardiology Governor's Award for Excellence twice; and was inducted as a Fellow of the Medtronic Bakken Society in 1980. He was voted the Institute of Electrical and Electronics Engineers (IEEE) Young Electrical Engineer of the Year in 1979. He has authored numerous publications including refereed articles in the *IEEE Transactions on Rehabilitation Engineering* journal; and holds several medical device pacing-related patents. In 1980, he was given Medtronic's "Invention of Distinction" award for his role as co-inventor of the tined pacing lead. Mr. Citron has served on many management committees and advisory boards, including the Biomedical Engineering Materials and Applications Roundtable and the National Research Council, from 2001 to present. Mr. Citron holds a M.S. in Electrical Engineering from the University of Minnesota.

Philip W. Miller currently serves as the Vice President of US Product Management in the Monsanto US Commercial Organization. He is responsible for product portfolio planning and execution throughout the product life cycle, including gathering and prioritizing product and customer requirements, defining the product vision, and working closely with research and development, corporate strategy, sales, marketing and support groups. Dr. Miller joined Monsanto in 1994 and has held numerous roles in the Monsanto Technology organization spanning Chemical Discovery and Biotechnology Research and Development. Key roles include Director of Biotechnology Crop Yield Enhancement and Crop Genomics research, Monsanto/Ceres Research Collaboration Lead, Director of the Monsanto Connecticut Research Center and Biotechnology Corn Pipeline Strategy Lead. Prior to his current role he served as Vice President of US Technology Development. Dr. Miller served on US Department of Agriculture, National Institute of Health, and National Science Foundation sponsored review panels, and as a scientific reviewer for numerous journals. He is the inventor on numerous patents and the author of seventeen scientific papers in journals including *Plant Molecular Biology* and book chapters. Dr. Miller earned his Ph.D. in Biochemistry and Molecular Biology from the University of Florida.

Lowell R. Nault is a Professor Emeritus at the Ohio State University**.** He has extensive research experience in the transmission of plant pathogens by arthropods and in the evolutionary biology of vectors and plant pathogens. He has worked in collaboration with US Department of Agriculture entomologists on a spectrum of problems ranging from molecular to landscape ecology, especially maize vectors and pathogens. Dr. Nault has authored well over 100 peer-reviewed articles and co-edited the text *The Leafhoppers and Planthoppers*. Dr. Nault is a fellow of several professional societies, including the American Phytopathological Society, the Entomological Society of America, and the American Association for the Advancement of Science. He served as president of the Entomological Society of America from 1990–1991. He was one of six Ohio State University faculty selected in 1999 as a "Distinguished Scholar." In 1982 and in 1985, he served as Program Manager for the US Department of Agriculture Competitive Research Grants Office (entomology, plant pathology, nematology, and weed science programs). From 1995 until he retired (2002), he served as Associate Director of the Ohio Agricultural Research and Development Center and as Interim Director in 1999. Dr. Nault received his Ph.D., with a major in Entomology and minor in Plant Pathology, from Cornell University.

MaryLou L. Polek currently serves as the Vice President for Operations of the California Citrus Research Board. She is responsible for implanting the industry-funded action program against the Asian citrus psyllid and Huanglongbing disease. As a plant pathologist who has specialized in citrus, she brings to the job more than 30 years' experience in plant disease diagnostics and laboratory management. She also serves on the executive committee of the California Huanglongbing Task Force as the Chair of the Science and Technology Advisory Committee. Dr. Polek has completed a 14-year tenure at the California Department of Food and Agriculture (CDFA) where she served in both the Integrated Pest Control and Emergency Projects branches. Most recently she served as program manager and plant pathologist for the Central California Tristeza Eradication Agency. Prior to her work with CDFA, Dr. Polek was a researcher at University of California, Riverside in the Plant Pathology and Botany and Plant Sciences departments, and earlier in her career she was an instructor for biology laboratory classes at Saddleback College. She has published in refereed scientific journals including *Phytopathology and Plant Disease*. She is a member of the American Phytopathological Society and the International Organization of Citrus Virologists. Dr. Polek earned her Ph.D. in Plant Pathology from the University of California, Riverside.

Howard-Yana Shapiro is the Global Director of Plant Science and External Research for Mars Incorporated**.** He leads the Mars global effort on the sequencing, annotation, and assembly of the *Theobroma cacao* genome. Dr. Shapiro is also an Adjunct Professor in the College of Agriculture and Environmental Sciences at The University of California, Davis and is the chair of the Advisory Board for the Agricultural Sustainability Institute. He has been involved with sustainable agricultural and agroforestry systems, plant genetics, and food production systems for over 40 years, with projects in the US, the EU, Mexico and Latin America, South America, and West Africa. He is a two-time Fulbright Scholar, a two-time Ford Foundation Fellow, and a winner of the National Endowment for the Humanities Award. Dr. Shapiro is formerly a university professor and for the last eleven years has been Vice President for Agriculture for Seeds of Change. Dr. Shapiro joined the International Assessment of Agricultural Science and Technology for Development as a Contributing Author on the Global Synthesis and co-wrote the chapter on Biotechnology. He was one of the founding members of the Sustainable Agriculture Roundtable, which was established in 2006. In 2007, he was awarded the Organic Leadership Award from the Organic Trade Association. In 2008, he was named a World Agroforestry Centre Fellow for his outstanding contribution to multifunctional agroforestry and global land-use issues.

Anthony M. Shelton is a Professor at the Department of Entomology at Cornell University's New York State Agricultural Experiment Station in Geneva, New York**.** Dr. Shelton is responsible for developing sound insect pest management strategies for vegetables, with spin-offs for other crops. Components of his program stress insect population ecology, biological control, plant resistance, agricultural biotechnology, insecticide resistance, insect movement, trap cropping, and plant productivity and marketability as a function of insect infestations. His program works with presently available strategies and helps incorporate them into pest management programs, and develops new strategies for the future. Most of Dr. Shelton's teaching responsibilities involve guest lectures on biotechnology, integrated pest management, and international agriculture. His international activities are focused primarily in India, China, and Latin America. He is a member of the Entomological Society of America, the Society for Invertebrate Pathology, and the National Agricultural Biotechnology Council. He has served on numerous panels, including as presenter to the 2002 NRC Committee on the Biological Containment of Genetically Modified Organisms and the 2007 US Agency for International Development Plant Biosafety Systems Review Panel. Dr. Shelton has been recognized with several awards, including the 2006 National Agriculture Extension Award for Publication, Organic Agriculture. Dr. Shelton has a Ph.D. in Entomology from the University of California, Riverside.

Lavern W. "Pete" Timmer is a Professor Emeritus at the University of Florida Citrus Research and Education Center (CREC). He is considered the leading citrus pathologist in the Americas and is known worldwide for expertise in plant pathology and citriculture. He has contributed substantially to the field of etiology, epidemiology, and control of fungal and bacterial diseases of citrus fruits and foliage while also studying other problems in citrus, such as blight, viral, and soil-borne diseases. Dr. Timmer held Associate Professor and Professor positions at the University of Florida-CREC from 1979 until his retirement in 2006. He is fluent in Spanish and was often invited to give presentations all over the world; he has also collaborated with other scientists on many international research projects. Dr. Timmer has been an Associate and Senior Editor of *Phytopathology* and *Plant Disease* journals, and was Senior Editor of the *Compendium of Citrus Diseases* and *Citrus Health Management*. He has published numerous articles in refereed journals and has authored or edited several books on citrus health. He received the International Organization of Citrus Virologists Special Award for Exceptional Research in 1989, the Citrus Research and Educational Scientist of the Year Award in 1995, the Lee Hutchins Award for excellence in research on fruit crops in 1996, and was inducted as a Fellow of the American Phytopathological Society in 2000. He received his B.S. in Botany and Plant Pathology from Michigan State University and his Ph.D. from the University of California, Riverside.

James H. Tumlinson, III is the Ralph O. Mumma Professor of Entomology and the Director for the Center for Chemical Ecology at Pennsylvania State University. He has done pioneering work on chemical communications between plants and insects, including volatile signals that attract natural enemies of insect pests of plants. He is a world leader in characterizing pheromones from diverse insect species and turning basic discoveries on chemical communications into novel insect management strategies. Dr. Tumlinson currently collaborates with scientists in Brazil, in Japan, and in South Africa to investigate plant-insect interactions and plant defenses. He focuses on the development of fundamental knowledge and principles that can be applied in environmentally safe pest management programs. Dr. Tumlinson has received numerous recognitions including membership to the National Academy of Science, 1997, the J.E. Bussart Memorial Award from the Entomological Society of America, 1990; The US Department of Agriculture Secretary of Agriculture's Award for Personal and Professional Excellence, 1995; and the Presidential Rank Award as a Meritorious Senior Professional in USDA, Agriculture Research Service, 2003. He was also the co-awardee of the 2008 Wolf Foundation Prize in Agriculture for the discovery of mechanisms governing plant-insect and plant-plant interactions. ISI Essential Science Indicators listed Tumlinson's publications in the top 1% in terms of total citations in the field of Environment/Ecology, 2002. He has published hundreds of refereed articles, conference papers, and reviews in journals such as the *Journal of Chemical Ecology* and the *Proceedings of the NAS.* Dr. Tumlinson earned his Ph.D. in Organic Chemistry from Mississippi State University.

Raymond K. Yokomi. Dr. Ray Yokomi has over 30 years of research experience in both applied and basic aspects of entomology, insect vectors, and plant pathology. He is recognized for his contributions in aphid transmission and characterizations of Citrus tristeza virus (CTV), detection and epidemiology of *Spiroplasma citri*, biological control of aphids, and plant-whitefly interactions and associated phytotoxemias. Dr. Yokomi joined the US Department of Agriculture (USDA), Agriculture Research Service in 1982 as an entomologist. Since 1997, his research has expanded to plant pathology, in particular the biological and molecular characterization of CTV and *S. citri*, the causal agent of citrus stubborn disease. Dr. Yokomi has served on various task forces in Florida and California on CTV and *Toxoptera citricida*, the brown citrus aphid. He is a member of the team that developed the Recovery Plan for Huanglongbing or Citrus Greening for the USDA Animal Plant Health and Inspection Service, National Plant Recovery System. He was a member of the Parent Committee on the Review of Citrus Greening Proposals in 2008. Dr. Yokomi has a Ph.D. in Entomology from the University of California, Davis.

Appendix C

Liaison Committee on Strategic Planning for the Florida Citrus Industry: Addressing Citrus Greening Disease

Calvin Arnold, US Department of Agriculture (USDA) Agricultural Research Service

Philip Berger, USDA Animal and Plant Health Inspection Service

Jackie Burns, Citrus Research and Education Center

Barbara Carlton*,* Peace River Valley Citrus Growers Association

Kristen Gunter, Macfarlane Ferguson & McMullen

John Jackson, Florida Citrus Industry Research Coordinating Council

Richard Kinney, Florida Citrus Packers Association

Peter McClure, Florida Citrus Production Research Advisory Council

Craig Meyer, Florida Department of Agriculture and Consumer Services

Bob Norberg, Florida Department of Citrus

Mike Sparks, Florida Citrus Mutual

Pete Spyke, Arapaho Citrus Management, Inc.

Tom Turpen, Technology Innovation Group

Gail Wisler, USDA Agricultural Research Service

Appendix D

Oral Presentations and Written Statements Submitted to the Committee

ORAL PRESENTATIONS

The following individuals made presentations to the Committee on Strategic Planning for the Florida Citrus Industry: Addressing Citrus Greening and the Disease.

May 15, 2009

Status of Citrus Greening and Its Impact on the Florida Citrus Industry
Peter McClure, Florida Citrus Production Research Advisory Council (FCPRAC)
Kristen Gunter, Florida Citrus Processors Association
Richard Kinney, Florida Citrus Packers
Mike Sparks, Florida Citrus Mutual
Peter Spyke, Indian River Citrus League

APHIS Citrus Disease/Pest Detection and Response Initiatives
Phil Berger, US Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS)

Florida Department of Agriculture and Consumer Services Citrus Health Response Program
Richard Gaskalla, Division of Plant Industry, Florida Department of Agriculture and Consumer Services

US Horticultural Laboratory Research Overview: Strategy and Breakthroughs
Calvin Arnold, US Horticultural Laboratory Research, USDA Agricultural Research Service (ARS)

USDA National Citrus Research Program Overview: Strategy and Breakthroughs
Gail Wisler, USDA-ARS Citrus Research and Education Center CREC
Research Overview: Strategy and Breakthroughs
Jackie Burns, USDA-ARS CREC

Roles and Relationships of Citrus Institutions in Florida
Tom Turpen, FCPRAC and Technology Innovation Group
John Jackson, Florida Citrus Industry Research Coordinating Council

July 28, 2009

Potential of Airborne Hyperspectral Imaging for Early Detection of Huanglongbing
Chenghai Yang, USDA-ARS
Kika de la Garza, Subtropical Agricultural Research Center

Possible Psyllid Control Approaches Derived from the Psyllid Transcriptome
Robert Shatters/Wayne Hunter, USDA-ARS, US Horticultural Research Laboratory

Potential Contributions of Citrus Genetics and Genomics to HLB Mitigation
Mikeal Roose, University of California, Riverside

New Ideas on Combating Huanglongbing
William O. Dawson, University of Florida (UF) Institute of Food and Agricultural Sciences (IFAS) Citrus Research and Education Center

Psyllid Control in Florida
Philip Stansly, UF-IFAS Southwest Florida Research and Education Center
Michael Rogers, UF-IFAS-Citrus Research and Education Center

Potential of Transgenic Psyllid for Sterile Insect Technique (SIT) or Interference with Transmission of Bacteria
David Lampe, Duquesne University

WRITTEN STATEMENTS:

The Potential Application of Female-Specific Insect Lethality for Psyllid Control
Luke Alphey, Oxford Insect Technologies

Research on Huanglongbing at Fundecitrus
Juliano Ayres, Fundecitrus

Possible Near Term Approaches to Psyllid Control
Charles Baer, Dupont Crop Production

Rational Management of Emerging Citrus Greening/ Huanglongbing Infections
Moshe Bar-Joseph, S. Tolkowsky Laboratory of Citrus Disease Research (Retired)

Psyllid Host Selection and Sensitivity to Soft Insecticides in Approaches to Psyllid Control; Huanglongbing Projects in Asia
Andrew Beattie, Center for Food and Plant Science, University of Western Sydney

Possible Near Term Solutions to Psyllid Control
John Bell, Bayer CropScience

Intellectual Property and Regulatory Approval of Transgenic Crops: New Challenges for the Public Research Sector in Fulfilling its Historical Role in Translational Agricultural Research
Alan B. Bennett, Public Intellectual Property Resource for Agriculture

Research Strategies for Insect-Vectored Plant Diseases
Donald A. Cooksey, University of California (UC) Riverside

Potential Strategies for Huanglongbing Management
Abhaya M. Dandekar, UC Davis

The Key Word to the Present Situation is "Urgency"
William O. Dawson, UF-IFAS-Citrus Research and Education Center

Huanglongbing Management in China
Xiaoling Deng, Citrus Huanglongbing Research Laboratory, Department of Plant Pathology, South China Agricultural University, and Jianchi Chen (Translator), USDA, ARS, Parlier, California

Possible Application of RNA Interference in Psyllid Control
Bryce W. Falk, UC Davis

Possible Near Term Approaches to Psyllid Control
Marc Fisher, Dow AgroSciences

Huanglongbing Projects in Brazil
Juliana Freitas-Astúa, Embrapa

Updated Assessment of Huanglongbing in Florida
Tim R. Gottwald, USDA-ARS

Soft Pesticides in Citrus to Maintain Best Possible Integrated Pest Management
Beth Grafton-Cardwell, UC Kearney Agricultural Center

Identification of Critical Gaps/Needs Addressing Solutions for Huanglongbing
Wayne Hunter, USDA-ARS

Possible Application of RNA Interference in Psyllid Control
Jin Hailing, UC Riverside

Potential of Transgenic Psyllid for SIT or Interference with Transmission of Bacteria
David J. Lampe, Duquesne University,

Potential of Landscape Management for Medium and Long-term Control of Psyllid in Citrus
Douglas A. Landis, Michigan State University

Psyllid Control: Chemicals, Methods and Time of Application in South Africa
Hennie F. le Roux and Tim G. Grout, Citrus Research International

Use of Psyllid Acoustics for Detection/Control of Psyllids (e.g Diaphorina citri)
Diana M. Percy, University of British Columbia

2009 Florida Citrus Pest Management Guide: Asian Citrus Psyllid and Citrus Leafminer
Michael E. Rogers, Phil A. Stansly and Lukasz L. Stelinski, University of Florida- IFAS

Quick Reference Guide to Citrus Insecticides and Miticides
Michael E. Rogers, Phil A. Stansly, James D. Yates and Lukasz L. Stelinski, University of Florida- IFAS

Potential Contributions of Citrus Genetics and Genomics to Huanglongbing Mitigation
Mikeal L. Roose, UC Riverside

New Insecticides as Part of a Near-term Approach for Psyllid Control and Insecticide Resistance Management
Nicholas Storer, Dow AgroScience

Potential of Airborne Hyperspectral Imaging for Early Detection of Huanglongbing
Chenghai Yang, USDA-ARS

Cucurbit Yellow Vine Disease
Astri Wayadande, Oklahoma State University

Appendix E

Significant Citrus Diseases Other Than Huanglongbing

Disease Name	Causal Organism/Vector	Distribution	Management/Control	Reference	Additional Information
Systemic Diseases					
Citrus Variegated Chlorosis	*Xylella fastidiosa* Transmitted by sharpshooters and other xylem-feeding leafhoppers	Southern Brazil; Argentina; Costa Rica	Propagation of healthy budwood; pruning infected limbs of newly-affected trees; removal of affected trees in young plantings.	Chang et al., 1993; Hartung et al., 1994; Timmer et al., 2003	Potentially important anywhere in the world.
Stubborn Disease	*Spiroplasma citri* Transmitted by several species of leafhoppers	Arid citrus regions, such as parts of California and Arizona, North Africa, and the Middle East	Avoiding infection in nurseries and young plantings.	Roistacher, 1991; Timmer et al., 2003	Unlikely to be a problem in humid areas where environmental conditions for spread do not exist.

Disease Name	Causal Organism/Vector	Distribution	Management/Control	Reference	Additional Information
Tristeza Decline and Stem Pitting	*Citrus Tristeza Virus* (CTV) Transmitted by several species of aphids	Asia, South America, South Africa, Australia, Spain, Israel, Southern Italy	Use of tolerant rootstocks and scion varieties; cross protection with mild, strains of CTV to delay infection/reduce severity of stem pitting.	Roistacher, 1991; Garnsey, 2005; Hilf, 2005; Costa and Müller, 1980	Decline strains spreading in the Mediterranean; the importance of stem pitting increases with spread of *Toxoptera citricidus,* the most efficient vector.
Citrus Sudden Death or Morte Subita dos Citros	Virus (member of Marafivirus) Graft transmissible; vector has not been identified	Brazil (southern Minas Gerais and northern São Paulo)	Use of tolerant rootstocks (such as Cleopatra and Sunki mandarins, Carrizo citrange, and Swingle citrumelo) instead of Rangpur lime.	Román et al., 2004; Bassanezi et al., 2003	Conditions outside of Brazil may not favor development of this disease.
Citrus Blight	Unknown Transmissible by root-piece and tree-to-tree root grafts	The Americas, Australia, South Africa	Replacement of affected trees with trees on tolerant rootstocks; no known cure for this disease.	Derrick and Timmer, 2000; Timmer and Bhatia, 2003	Most important in Florida and Brazil; potential for development elsewhere uncertain.
Leprosis	Bacilliform Virus Transmitted by *Brevipalpus* mites	South America and Central America	Control of its mite vector using acaricides.	Roistacher, 1991; Bastianel et al., 2006	Only locally systemic in infected areas of the tree.
Witches' Broom Disease of Lime (WBDL)	Phloem-limited phytoplasma May be transmitted by *Hishimonus phycitis* leafhoppers	Oman; United Arab Emirates; Iran	WBDL is only confined to limes; sweet orange, mandarin and grapefruit are apparently resistant.	Garnier et al., 1991; Roistacher, 1991	Dangerous disease for western Mexico and other producing areas of Mexican lime.

Disease Name	Causal Organism/Vector	Distribution	Management/Control	Reference	Additional Information
Viroids	Citrus exocortis viroid (CEVd)**;** Cachexia CVdI, CVdII; CVdII, CVdIV	Wherever citrus is grown	Eliminated by certification programs; sterilization of pruning tools and budding knives.	Duran-Villa et al., 2000	Have been largely eliminated by budwood certification programs; important primarily on rootstocks such as trifoliate orange and its hybrids and Rangpur lime.
Bacterial and Foliar Fungal Diseases					
Citrus Canker	*Xanthomonas citri* subsp. *citri*	Asia; southern Brazil, Argentina; Uruguay; Caribbean; Florida	Quarantine and eradication where not endemic; in endemic areas, windbreaks to reduce severity and copper products to prevent fruit infection.	Civerolo, 1984; Koizumi, 1985; Graham and Gottwald, 1991; Gottwald et al., 2001; Schubert et al., 2001; Graham et al., 2004	Not only important for fresh fruit; causes significant leaf and fruit drop.
Citrus Black Spot	*Guignardia citricarpa* (Anamorph: *Phyllosticta citricarpa*)	Widespread in the humid to semi-arid citrus-growing areas in the Southern Hemisphere	Controlled primarily by fungicide applications (benzimidazoles, copper products, dithiocarbamates, or strobilurin) in the summer.	Peres and Timmer, 2003; Timmer et al., 2004;	Not only important for fresh fruit; causes significant fruit drop as well.
Pseudocercospora Fruit and Leaf Spot	*Pseudocercospora anglonensis*	Angola; Mozambique; Yemen; Western and Southern Africa	Preventive applications of copper fungicides, chlorothalonil, flusilazole, or propineb.	Seif and Hillocks, 1997; Timmer et al., 2004	Not only important for fresh fruit; causes significant leaf and fruit drop.

Disease Name	Causal Organism/Vector	Distribution	Management/Control	Reference	Additional Information
Greasy Spot	*Mycosphaerella citri* and other *Mycosphaerella* spp.	Uncertain; widespread in the Caribbean area; some problems occur elsewhere and may due to other species of *Mycosphaerella*.	Foliar applications of petroleum oils or various fungicides.	Mondal and Timmer, 2006	May cause severe defoliation and fruit blemishes, especially on grapefruit and lemons.
Sweet Orange Scab	*Elsinoe australis*	South America	Application of sterol-inhibiting fungicides, ferbam, benzimidazoles, strobilurins, or copper products.	Timmer et al., 2004	Only important for fresh fruit production.
Postbloom Fruit Drop	*Colletotrichum acutatum*	Western Hemisphere	Application of fungicide such as benzimidazoles, strobilurins, captan, maneb, or ferbam. A computer assisted system has been developed for timing fungicide applications.	Timmer et al., 1994; Peres et al., 2005 ; Timmer and Brown, 2000	Only important where rainfall is high during bloom.
Alternaria Brown Spot	*Alternaria alternata*	First described in Australia in 1903; Mediterranean; Caribbean Basin; South Africa; South America	Minimize leaf wetness (avoiding overhead irrigation); use of disease-free nursery trees; avoiding excessive nitrogen fertilization and irrigation; application of foliar fungicides. alter-rater, a predictive system, has been developed to time sprays.	Timmer et al., 2003; Akimitsu et al., 2003	Only affects some tangerines and hybrids; spreading in semi-arid areas.

Appendix F

Significant Citrus Insect and Mite Pests

Insect Name	Distribution	Management/Control	Reference	Additional Information
Mediterranean Fruit Fly (*Ceratitis capitata*)	Asia, Africa, Europe, Australia, South America; introduced into the US but successfully eradicated	Insecticide-treated baits applied to scattered trees in the orchard; most Medfly larvae are killed when fresh fruits are shipped under cold storage	Thomas et al., 2007; Lanza et al., 2005	Serious pest in many areas
Oriental Fruit Fly (*Bactrocera dorsalis*)	Asia; occasionally found in Florida and California	Similar to Mediterranean fruit fly	Weems et al., 2008	Most serious of all fruit flies, except for Mediterranean fruit fly
Mexican Fruit Fly (*Anastrepha ludens*)	Mexico; Central America; Frequently found in Texas	Similar to Mediterranean fruit fly	Weems et al., 2008	Less serious than other fruit flies

Insect Name	Distribution	Management/Control	Reference	Additional Information
Caribbean Fruit Fly (*Anastrepha suspensa*)	Caribbean Basin; Commonly found in Florida	Similar to Mediterranean fruit fly	Weems et al., 2008	Less serious than other fruit flies
Diaprepes Root Weevil (*Diaprepes abreviatus*), aka West Indian Sugar Rootstock Borer Weevil	Caribbean; Common in Florida; Found but not widely distributed in Texas and California	Foliar insecticide applications to kill adults; soil-applied insecticides to kill larvae; parasitic nematodes applied to soil surface to kill larvae in the soil; these measures are not highly effective in controlling the damage	McCoy et al., 2009; Woodruff, 1985	Most serious root weevil pest
Blue-green Root Weevils (*Pachneus* spp.)	Limited distribution in the Caribbean Basin and Florida	Insecticide applications to control adult weevils	McCoy et al., 2009; Woodruff, 1985; Hall, 1995	Feeds primarily on fibrous roots causing minor to moderate yield loss
Little Leaf Notcher (*Artipus floridanus*)	Limited distribution in the Caribbean Basin and Florida	Insecticide applications to control adult weevils	McCoy et al., 2009; Woodruff, 1985; Hall, 1995	Feeds primarily on fibrous roots causing minor to moderate yield loss
Fuller's Rose Beetle (*Asynonychus godmani*)	Occurs widely on many hosts, but is of little significance as a citrus pest	Insecticide applications to control adult weevils	McCoy et al., 2009; Woodruff, 1985; Hall, 1995	Feeds primarily on fibrous roots causing minor to moderate yield loss

Insect Name	Distribution	Management/Control	Reference	Additional Information
Expothalmus spp. .	Limited distribution in the Caribbean Basin; not in Florida	Insecticide applications to control adult weevils	McCoy et al., 2009; Woodruff, 1985; Hall, 1995	Attacks the crown of the tree in addition to roots and can kill trees
Citrus Leafminer *Phyllocnistis citrella*	Widespread in citrus	Biological control and insecticide applications to young flush	Heppner, 1993	Exacerbates the severity of citrus canker
Brown citrus aphid *Toxoptera citricida*	Asia, Pacific Islands, Australia, New Zealand, Africa, South. America, Central America, Eastern Mexico, Florida, northern Portugal and northern Spain	Biological control; insecticide application usually not beneficial	Halbert et al., 2004	Important primarily as a vector of *Citrus tristeza virus*
Citrus thrips *Scirtothrips citri*	Northern Mexico and western US	Application of insecticides	Kerns, et al., 2001	Superficial damage; important only for fresh fruit
Sharpshooters *Homalodisca vitripennis* (=*H. coagulata*)	Southeastern US, Florida, California, Mexico	Biological control; in California, insecticides used against overwintering adults	Blua and Morgan, 2003	Minor pest; important vector if citrus variegated chlorosis is introduced to California
Brevipalpus mites *B. californicus* *B. obovatus* *B. phoenicis* *B. lewisi*	Tropical and subtropical distribution but occur with citrus worldwide	Biological control and acaricides and oil	Childers et al., 2001	Minor pest; feeds on fruit and blemishes rind; important because some species transmit leprosis

Appendix G

Missions of University, Government, and Non-government Agencies with Responsibilities Related to Citrus Production and Marketing

Type	Institution/Agency/Organization	Mission
University	**University of Florida-Institute of Food and Agricultural Sciences (UF-IFAS)**	
	Various Departments (Gainesville, FL)	Develop knowledge in agriculture, human and natural resources, and the life sciences. Enhance and sustain the quality of human life by making that information accessible
	Indian River Research and Education Center (IRREC)	Evaluate citrus cultivars and citrus production, provide extension service
	Citrus Research and Education Centre (CREC)	Discover and deliver innovative solutions that empower citrus to conduct responsible and profitable business
	Southwest Florida Research and Education Center (SWFREC)	Conduct research and extension in citrus horticulture, physiology, entomology, plant pathology, and economics

Type	Institution/Agency/Organization	Mission
Federal, State or Local Government	**United States Department of Agriculture-Agricultural Research Service, (USDA-ARS)**	Conduct research to develop and transfer solutions to agricultural problems of high national priority
	US Horticultural Research Laboratory (USHRL) Fort Pierce, FL	Conduct research to develop new approaches to controlling insects in subtropical fruit, vegetable, and ornamental crops
	Foreign Disease-Weed Science Unit, Ft. Detrick, MD	Focus on new and emerging plant pathogens that pose a threat to American agriculture and must be kept under containment
	US Citrus and Subtropical Products Laboratory, Winter Haven, FL	Conduct research in food science and postharvest areas for fresh and processed tropical, subtropical fruits, and vegetables
	Crop Diseases, Pests and Genetics Research Unit, Parlier, CA	Focus on insect-vectored diseases of citrus and grapevine, especially *C*Las genetic diversity and gene expression
	National Clonal Germplasm Repository for Citrus & Dates, Riverside, CA	Improve collections of citrus genetic resources, including more rapid and efficient characterization, conservation and propagation of material and detection of graft-transmissible pathogens
	Kika de la Garza Subtropical Agricultural Research Center, Weslaco, TX	Conduct research on citrus and vegetable processing including integrated pest management (IPM), biological control, pesticide resistance, and remote sensing

Type	Institution/Agency/Organization	Mission
	US Department of Agriculture-Animal and Plant Health Service, (USDA-APHIS)	Safeguard crop agriculture and natural resources from the risks associated with the entry, establishment, or spread of animal and plant pests and noxious weeds, with the intent of ensuring an abundant, high-quality, and varied food supply
	CPHST (Center for Plant Health Science and Technology)	
	PPQ (Plant Pest and Quarantine)	
	CHRP (Citrus Health and Recovery Program)	Protect citrus production by establishing minimum standards for citrus inspection, regulatory oversight, disease management, education and training
	Florida Citrus Production Research Advisory Council (FCPRAC)	Devote grower tax funds up to 1 cent per box of commercial citrus for research focused on various aspects of the industry
	Florida Department of Citrus (FDOC)/Florida Citrus Commission	Provide marketing, research, and regulatory support for Florida citrus products. Manage and act on the advice of its advisory committees
	Florida Department of Agriculture and Consumer Services- Division of Plant Industry (FDACS-DPI)	Detect, intercept, and control plant and honey bee pests that threaten Florida's native and commercially grown plants and agricultural resources
	Citrus Administrative Committee (CAC)	Determine grade, size, maturity, quality, and volume regulations on Florida citrus
	Citrus Research and Development Foundation (CRDF)	Coordinate citrus disease researchers, managing contracts, detecting and reducing overlaps in research projects where appropriate, assessing progress and addressing intellectual property and other commercialization and product development issues

	Institution/Agency/Organization	Mission
	US Department of Labor (DOL)	Promote the welfare of the job seekers, wage earners, and retirees of the United States
	US Environmental Protection Agency (EPA)	Protect human health and the environment
	US Food and Drug Administration (FDA)	Protect and advance the public health
	US Immigration and Customs Enforcement (ICE)	Protect America and uphold public safety by targeting the people, money and materials that support terrorist and criminal activities
	US Occupational Safety and Health Administration (OSHA)	Prevent work-related injuries, illnesses, and deaths
	Water management districts	Administer flood protection programs, perform technical investigations into water resources, develop water management plans for water shortages, acquire and manage lands for water management purposes. Manage programs to manage water consumption, aquifer recharge, well construction and surface water management.
	Florida Citrus Industry Research Coordinating Council (FCIRCC)	Assure that the most pressing research needs facing the industry are addressed in an efficient manner
Non-government Organization	**Florida Citrus Packers (FCP)**	Solve problems members encounter and to secure cooperation between producers and shippers in marketing of fresh citrus and gift packs
	Florida Citrus Processors Association (FCPA)	Represent, communicate, protect, and enhance the interests of its members. Promote the growth and welfare of the citrus industry

Florida Citrus Mutual (FCM)	Help Florida citrus growers produce and market their crops at a profit
Florida Citrus Production Managers Association (FCPMA)	Serve as an educational clearinghouse for the production managers through seminar presentations
Florida Foundation Seed Producers, Inc. (FFSP)	Negotiate exclusive and non-exclusive license agreements for cultivars of citrus and other Florida crops
Florida Gift Fruit Shippers Association (FGFSA)	Foster communication and education among producers and shippers of high quality specialty fruit
Florida Nursery, Growers and Landscape Association (FNGLA)	Enhance its members' business success through political leadership, education, marketing, research, certification and services
New Varieties Development and Management Corporation (NVDMC)	Provide Florida growers affordable and timely access to new citrus varieties
Regional grower associations	Handle local issues such as water management, community relations and local planning

Appendix H

Citrus Bacterial Canker: Outbreaks and Regulatory Response

Citrus bacterial canker (CBC), caused by *Xanthomonas citri* subsp. *citri* (syn. *X. campestris* pv. *citri*, *X. axonopodis* pv. *citri*) has been introduced into Florida several times. Below is a brief history of the detection of CBC in Florida and the responses mounted by the Florida Department of Agriculture and Consumer Services Division of Plant Industry (FDACS-DPI) and US Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS).

1910: Trifoliate rootstock infected with the Asian or "A" strain of the bacterium were brought into Florida from Japan. The response to CBC detection included the implementation of a quarantine to prevent the movement of citrus plants, the destruction of over three million nursery plants, and the removal of over a quarter million field trees. Eradication was declared a success in 1933 and regulatory detection surveys have continued to the present day since this initial CBC outbreak.

1984: An outbreak of citrus bacterial spot, caused by a less virulent strain ("E" strain) (now *X. alfalfae* subsp. *citrumelonis*) was detected on nursery stock. Over twenty million citrus plants were destroyed.

1986 to 1994: The "A" strain was detected in residential citrus in the Tampa Bay area and in nearby commercial groves. For the first time, not only were infected trees destroyed but also all exposed host plants within 38 m (125 ft). Also for the first time, residential trees were eradicated. After the destruction of 600 residential trees and almost 90,000 production trees, and no further detections for a two-year period, an official declaration of successful eradication was made in 1994. Regulations were suspended.

1995: The "A" strain of the CBC agent was detected on residential citrus in the vicinity of the Miami International Airport. It is believed that the appearance of CBC in commercial citrus resulted from spread out of residential sources. An eradication program was initiated immediately, and quarantine regulations were re-instated. The protocol required removing or buckhorning (removal of all green aboveground tissue, also known as hat-racking) all potential hosts within 38 m (125 ft) of an infected tree. After one year, the infection level remained too high to continue with the buckhorn protocol and it was suspended. In late 1996, all exposed trees had to be removed. Resources were quickly depleted; the program was under-staffed, the quarantine area rapidly expanded to 93,499 ha (361 mi^2). The situation was exacerbated by the detection of CBC in another area in west central Florida. The isolate from this area was found to be genetically identical to the isolate responsible for the 1984 outbreak, which indicated that CBC had not been successfully eradicated after all. In early 1998, the Florida Commissioner of Agriculture and the Citrus Canker Eradication Program (CCEP) declared a moratorium on the removal of exposed citrus trees because a judge stopped tree removal in response to homeowner lawsuits using standards in effect at the time. Homeowners were opposed to removal of trees that were not infected without evidence that they could be sources of inoculum. During the moratorium, the bacteria quickly spread and additional sites were discovered in southwest Florida. During this 12-month study period, the 1,900 ft rule (580 meter radius) was created which expanded the quarantine to 129,500 h (500 mi^2) with a program budget of $9.5 million. By 2000, the Persian lime industry in south Florida had been destroyed (Schubert et al., 2001).

2004: The three hurricanes and a tropical storm that crossed the Florida peninsula dispersed the pathogen even further across the state (Irey et al., 2006a; Gottwald and Irey, 2007); resulting in the establishment of new infections at substantial distances from the known existing infections. Survey and eradication efforts by the FDACS and USDA-APHIS became even more intense to eliminate new outbreaks before the next hurricane season.

The FDACS and USDA-APHIS simultaneously employed the following survey protocols: the sentinel grove survey (based on sentinel commercial blocks of susceptible cultivars), the targeted grove survey (based on meteorological data that used a GIS-weather model to predict the direction of disease spread due to hurricanes), the delimiting grove survey (which was performed once new infections were discovered) and the self survey (performed by the grower). Production managers and other workers that are in contact with commercial citrus on a daily basis were trained by a joint University of Florida and FDACS-DPI program to search for, recognize, and report suspicious symptoms to FDACS-DPI or USDA-APHIS inspectors for confirmation.

Not all citrus canker-infected trees detected in 2004 had been removed by the time the first hurricane (Hurricane Wilma) hit the Florida peninsula in October 2005. Of the 80,000 acres of commercial citrus identified as affected after the 2004 season, 32,000 acres remained even with the removal of 120,000 trees per week. It was estimated that an additional 168,000 to 220,000 acres of commercial citrus would have to be destroyed after the 2005 hurricane season under the 1900-foot protocol. At this point in time, the annual federal appropriation to maintain the program was $36 million. Eradication costs significantly increased from $10 million in 1996 to $50 million in 1999. In 2000, program costs escalated to $145 million. A panel of USDA scientists and global experts in citrus diseases conferred and concluded that the disease was now so widely distributed that eradication was not feasible.

On January 11, 2006 the USDA issued a press release stating that it would discontinue all efforts to eradicate canker. Subsequently, the Florida House of Representatives decided to halt the eradication campaign and repeal the 1900-foot protocol on May 3, 2006. However, because of the value of the fresh fruit industry and not every citrus production area in Florida was severely infected, there was still considerable support from the production industry to continue with a revised management program. Hence, the concept of a State-Federal-Commercial Industry Citrus Health Response Plan was launched. It is based on best management practices, living with the disease while minimizing production losses, and the implementation of a quarantine that prohibits the movement of fruit from known-infected areas of the state to non-infected areas and to other citrus-producing states. This plan has evolved into a National program, the Citrus Health Response Program, and is administered by the USDA-APHIS. USDA-APHIS has recently lifted the quarantine on Florida fresh fruit after findings that commercial fruit is not an important means of spread of the disease, thus allowing growers more flexibility in marketing their fruit.

Factors contributing to the failure of the Canker Eradication Program include:

- Time required for tree removal: A few homeowners refused to comply with tree removal or provide access to residential properties, and initial resources (manpower, vehicles, and funding) were limited.
- While legal battles for tree removal were being fought, inoculum sources remained during the declaration of moratorium in 1998 and the disease spread to new areas of the state through human movement.
- Tropical storms and hurricanes were optimal for bacterial spread (bacterial cells blown long distances by wind and carried in rain drops).
- Florida lacks geographical barriers such as mountains.
- Lack of a positive public relations campaign especially in ethnic residential areas.

Lessons for huanglongbing (HLB) learned from the canker experience:

- Importance of disease-free sources of citrus propagation material and nursery stock.
- Eradication actions taken at the first detection of Asian citrus psyllid (ACP) in 1998 might possibly have slowed ACP spread and delayed HLB's incursion (although ACP control efforts elsewhere have not been very successful).
- Intensive surveys for the *Candidatus* Liberibacter asiaticus pathogen, if conducted in 1998, might have stimulated action to slow the spread of HLB.
- Effective public outreach campaigns help to make the urban sector part of the solution to maintaining the commercial industry.
- While plant pathogens and their vectors and insect pests are part of biology, their implications enter the realm of social and political arenas. The urban sector does not always trust scientists or regulatory personnel. These should be taken into consideration when developing a public outreach campaign.
- Massive amounts of funding do not always solve the problem.

Appendix I

Detection of Asian Citrus Psyllid and Huanglongbing in Florida and Major Events and Activities That Occurred in Response to Their Detection (June 1998–August 2009)

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
1998 June	The Asian citrus psyllid (ACP) was detected by the Florida Department of Agriculture and Consumer Services-Division of Plant Industry (FDACS/DPI) in Palm Beach County, FL;	Surveys for huanglongbing (HLB) were also done by FDACS-DPI personnel in Palm Beach County where the ACP was first found	ACP was detected in Florida, distributed along Highway 1 on the east coast of Florida, from Broward to St. Lucie counties and was apparently limited to dooryard host plantings at the time of its discovery	http://www.doacs.state.fl.us/press/2005/09022005_2.html
1999-2000	Surveys were conducted after an erroneous report that HLB was found in FL	Surveys were done in Homestead (Miami-Dade County), West Palm Beach (Palm Beach County), and Ft. Pierce (St. Lucie County)	HLB was not found by the end of 2000; Initial surveys indicated that ACP population probably was not contaminated with HLB	Halbert et al., 2008
2001	ACP was found in 31 counties in FL			http://entomology.ifas.ufl.edu/creatures/citrus/acpsyllid.htm
2003-2005 (spring)	Intensive surveys were conducted in Orlando and	Surveys performed by Cooperative Agricultural	These surveys were done to detect HLB, but the disease was not found until August 2005	Halbert et al., 2008

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
	Tampa, FL	Pest Survey (CAPS) teams		
2003-2005	Informational Talks on ACP	Presented by the National Plant Diagnostic Network (NPDN)	Between 2003 and 2005, 19 recorded presentations were viewed by 349 registered attendees from California, Texas, and Florida. The primary audience for training consisted of the following groups: county extension agents; state and/or federal survey specialists; state and/or federal inspectors; consultants; growers; and various private industry personnel. Registered First Detectors throughout the US also receive the monthly NPDN First Detector newsletter, which periodically includes information about target pests such as citrus greening and ACP.	Amanda Hodges, personal communication
2004	Taxonomic Training for Entomologists	Conducted by the NPDN; ACP was covered in the training	Extensive taxonomic training was provided to entomologists in the southern US region. Helped entomologists in the southern region to screen for ACP. One of the entomologists (from LSU AgCenter) who attended the 2004 Workshop was involved in the initial detection of ACP in Louisiana in 2008	http://conference.ifas.ufl.edu/homoptera Amanda Hodges, personal communication
2005 August	HLB was discovered in Miami-Dade County, FL	United States Department of Agriculture (USDA) and FDACS CAPS (Cooperative Agricultural Pest Survey) discovered the disease	The 2005 survey and another survey in January-February 2006 indicated that by that time HLB was discovered in FL, it had spread extensively and could not be eradicated	http://www.doacs.state.fl.us/pi/caps/surveys.html; http://www.doacs.state.fl.us/press/2005/09022005_2.htmlHalbert et al., 2008

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
September	Citrus Greening Briefing Session held in Lake Alfred, FL	Initial effort following the detection of HLB in Miami-Dade County.	The briefing session was conducted to gather more information about the disease situation so that preliminary decisions can be made; Research needs were also identified at this meeting; Attended by representatives from USDA Animal and Plant Health Inspection Service (APHIS), Plant Pest and Quarantine (PPQ), Center for Plant Health Science and Technology (CPHST), University of Florida (UF), Citrus Research and Education Center (CREC), and FDACS-DPIURL: http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/twg/attendees.pdf	http://www.doacs.state.fl.us/pi/chrp/greening/researchneedsgoals.pdf; http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/twg/minutes9-8-05.pdf; http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/twg/researchneedsgoals.pdf
November	2nd International Citrus Canker and HLB Research Workshop was held	Hosted by Florida citrus mutual (FCM) and co-organized by USDA ARS (Tim Gottwald), UF, USDA APHIS, and FDACS	Invited scientists and participants were charged with developing a prioritized list of research recommendations. The 2005 meeting was attended by about 150 international scientists.	http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/twg/2ndcc-hlb-workshop.pdf T. Gottwald, personal communication

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
2006 January	FL HLB Science Panel Meeting	Organized by USDA/APHIS and the FDACS/DPI	The Science Panel was created in order to provide guidance to state and federal officials for an HLB response that is based on sound science; The panel concluded that the disease was already too widespread to attempt eradication	http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/twg/hlbsciencereport1-31-06.pdf T. Gottwald, Meeting Abstract, Proc of the International Workshop on Citrus Greening 2006
April	Florida Citrus Production Research Advisory Council (FCPRAC) votes to spend all available monies on HLB (with some on canker)		Council decides that HLB and canker are big enough threats to warrant additional funding	Peter McClure, personal communication; Marshall Wiseheart, personal communication
	Citrus Health Response Program was implemented by the FDACS-DPI		Program created to support the industry through training, education, and outreach	http://www.doacs.state.fl.us/pi/chrp/index.htmlR. Gaskalla, personal communication
October	A Recovery Plan for HLB or Citrus Greening was developed	Developed by National Plant Disease Recovery System (NPDRS) of APHIS		http://www.ars.usda.gov/SP2UserFiles/Place/00000000/opmp/CitrusGreening61017.pdf
October	Southern Gardens receives first sample for HLB testing (free of charge)	The Southern Gardens HLB diagnostic clinic was established in order to provide service to the industry that the federal and the state did not have the capacity to provide	Over 112,000 grower samples have been run so far, in addition to 18,000 samples from Southern Gardens itself	Mike Irey, personal communication

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
November	Announcement of the formation of two research task forces to deal with HLB and canker by the Florida Department of Citrus (FDOC)	The Council (FCPRAC) asked the UF Institute of Food and Agricultural Sciences (IFAS) and USDA scientists to concentrate efforts on HLB and canker	FCPRAC gets additional funding from the State of Florida and the FDOC; The Council cooperates with California Citrus Board (CRB) to sponsor five projects	Marshall Wiseheart, personal communication
2007 January	Greening and Canker Field Day	UF-IFAS Extension	This was conducted so that participants can see firsthand the symptoms and effects of citrus canker and greening on a commercial grove	http://citrusagents.ifas.ufl.edu/newsletters/atwood/Citruslines%20December%202006.pdf
	Training Module on Citrus Greening posted at NPDN website	This module was part of the NPDN Special Topic Training Modules	Available for educators to use at the 144 recorded NPDN First Detector training sessions that have occurred since the release of the presentation in December 2007. Presentation may also be used by those not conducting official First Detector training.	http://www.npdn.org/DesktopDefault.aspx?tabindex=3&tabid=52 Amanda Hodges, personal communication
	Approximately $1 million funding was allotted for HLB/canker research			Peter McClure, personal communication
March	2007 HLB Technical Working Group (TWG)-conference call	USDA APHIS PPQ convened a meeting of the HLB TWG on March 13, 2007	The purpose of the conference was to make recommendations on survey and regulation of HLB; The HLB TWG met because of: 1) potential detections of the HLB pathogen in its insect vector in Florida and Texas, and 2) to discuss experimental evidence characterizing the host status of *Murraya* spp.	http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/twg/2007%20HLBconf-call.pdf

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
May	Invasive Arthropod Workshop	Organized by the NPDN	This workshop also covered the ACP and citrus greening; The aim was to increase knowledge of exotic pests and diseases. Intensive hands-on training was also provided. Approximately 70 cooperative extension personnel, diagnosticians, inspectors, and survey specialists from around the southern region of the US attended. Conference abstract proceedings available at: http://insectscience.org/9.61/	http://conference.ifas.ufl.edu/arthropod/ Amanda Hodges, personal communication
June	The Council (FCPRAC) met to determine funding of 82 research projects for over $5M	Total funding includes: FDOC pledge $2M, FCPRAC funds $1.6M to match $3.75M from Legislative Appropriation. $250,000.00 also appropriated for UF to develop an diagnostic lab to test samples for HLB		Marshall Wiseheart, personal communication
December	USDA APHIS Citrus Greening Summit (National Plan Development)	Findings released April 2008; http://www.flcitrusmutual.com/files/9bec29bf-5dc1-4349-b.pdf	The Summit was convened to share and gather the latest information on the epidemiology of citrus greening and to discuss industry, State, and USDA strategies and activities to address the disease and its insect vector, the ACP. A total of 65 sector leaders attended, representing USDA agencies, State regulatory agencies in citrus-producing States, research institutions, and citrus and nursery industry-based organizations.	http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/citrusgreening_summit_plan.pdf
2008 January	FCPRAC decides to create a scientific review panel after consultation with Steve Lindow			Marshall Wiseheart, personal communication

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
January	The Florida Citrus Industry Research Coordinating Council (FCIRCC) Greening Task Force forms the Greening Research Oversight Committee		To start the development of a comprehensive Research Management Plan	Bob Norberg, personal communication
January	Sale of infected *Murraya paniculata* was prohibited by the state on Florida	New regulations required that all *Murraya paniculata* plants to be obtained from clean sources and produced under screen; Regulations also require *Murraya* plants to be treated with systemic pesticide prior to sale		Halbert et al., 2008
January	Florida statues enacted the CHRP recommendation for nursery tree production	According to this regulation, all certified nursery trees have to be grown within protected structures	Regulations were designed to prevent planting of HLB and canker infected stock in commercial groves	Halbert et al., 2008
February	The Southwest Florida Research and Education Center (SWFREC) HLB Diagnostic Laboratory began its operation	Established through the efforts of the Gulf Citrus Growers Association (GCGA) and elected officials; Growers, elected officials and industry reps convinced the Florida legislature to appropriate $250,000 in state funds to establish the HLB Lab in Immokalee	Established to serve grower and researcher HLB diagnostic needs; By Sept. 2008, the HLB clinic has processed 4,000 samples. By May 2009, the lab has received over 12,000 grower samples, approximately 4000 research samples, and 700 screen house samples from the Budwood facility in SWFREC	http://www.imok.ufl.edu/hlb/ Pamela Roberts, personal communication

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
	USDA National Program and NAS assistance sought and received by FDOC/FCIRCC/FCPRAC	FDOC met with the National Academy of Sciences (NAS) and USDA Agricultural Research Service (ARS) in Washington, D.C.	To suggest the formation of an Expert Panel (NAS/National Research Council) and SWAT-Team (ARS)	Peter McClure, personnal communication; Bob Norberg, pers.comm.
	FDOC pledges $20 million—legislature pledges $2 million, FCPRAC has $2 million—$24 m available for research			Peter McClure, personal communication
April	Greening Summit	Sponsored by UF-IFAS Extension and the FCPRAC	Topics covered: status of HLB, research projects, control programs, sample submission, survey techniques; There were also grower panels and a discussion of the Brazilian experience	http://citrusagents.ifas.ufl.edu/events/greening_summit/
April	National Research Council (NRC)/NAS Citrus Greening Meeting of Experts	Experts from various disciplines convened in Ft. Pierce and West Palm Beach, FL	HLB research priorities identified by NRC panel	http://southeastfarmpress.com/news/citrus-greening-0610/
April	USDA ARS Citrus Greening Workshop		HLB research priorities identified by USDA ARS panel	http://southeastfarmpress.com/news/citrus-greening-0610/
June (up to present)	Technology Innovation Group (TIG)/Tom Turpen hired by FCPRAC as professional program manager		FCPRAC engaged TIG for research portfolio management, with Tom Turpen serving as Program Manager	Peter McClure; Tom Turpen, personal communication
June	Request for Proposals (RFP) was announced by the FCPRAC through the NAS	RFP based on HLB Research Priority List created by NRC/NAS		http://www.fcprac.com/submit-guidelines-2008.pdf

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
June	FL Rep Matt Hudson visited SWFREC	The visit was facilitated by Collier County Cooperative Extension director Robert Halman	Rep Matt Hudson's visit included learning about Citrus/HLB research programs: propagation of citrus trees under cover of two screen house structures, psyllid research being conducted in a glass greenhouse and the HLB Lab currently testing citrus samples for HLB	SWFREC Newsletter
August	USDA CPHIST HLB TWG Meeting	Report Released Sept. 2008	The HLB TWG was asked to provide scientifically-based recommendations to decision makers for at-risk regions and industries to try to best prepare for the arrival and establishment of the ACP where it is not yet present or establishment and spread of HLB where ACP is currently established; HLB TWG was asked to develop options on a regional basis	http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/twg/HLB%20TWG%20Report%20Final%20NOLA%20080808.pdf
September-November	Submission and Review of Research Proposals	Proposal review conducted by the NRC	236 pre-proposals and 205 full proposals were received in response to the RFP	
September	TWG on Commercial Production and Movement of Citrus Nursery Stock from FL to non-citrus producing states-Findings and Recommendations	APHIS		http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/twg/cns-twg-report.pdf
September	FL citrus growers visited central CA	Hosted by the California CRB	To coordinate research activities and educational opportunities	John Jackson's Progress Report to FCPRAC Sept. 30, 2008

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
September-October	Florida Mini-Greening Summit was held	Held in Lake County, Highlands County, Hendry County, DeSoto County, Polk County, and St. Lucie County. Presented by the FL Cooperative Extension Service Citrus Extension Agents		http://desoto.ifas.ufl.edu/pdf/Citrus%20Newsletters/Citrus%20PDF%20Documents/Florida%20Mini-Greening%20Summit.pdf
October	USDA Awards more than US$28 million through the Specialty Crop Research Initiative (SCRI)	USDA/ARS Kika de la Garza Subtropical Agricultural Research Center (TX) was awarded $493,290 for the project Development of an Area-Wide Approach for Controlling Infection and Spread of HLB of Asian Citrus Psyllid	The Specialty Crop Research Initiative was established by the 2008 Farm Bill to support the specialty crop industry	http://www.environmental-expert.com/resultEachPressRelease.aspx?cid=31108&codi=38774&lr=1
October	Business Plan created by Technology Innovation Group (TIG) was approved by FCPRAC			Tom Turpen, personal communication
November 17	*Candidatus* Liberibacter asiaticus was removed from the PPQ List of Select Agents and Toxins	*Candidatus* Liberibacter africanus was also removed from the PPQ List of Select Agents and Toxins; *Candidatus* Liberibacter americanus was not added to the list	Delisting of *C*Las meant that grower funds did not have to be used to upgrade both IFAS and USDA labs in Florida; before *C*Las was delisted, many other scientists in other locations couldn't work on HLB, now more scientists can conduct research on HLB.	http://www.fas.org/sgp/news/2008/10/select.html; http://edocket.access.gpo.gov/2008/E8-23887.htm P. McClure, personal communication

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
November	Non-profit organization, Advanced Citrus Technology Group (ACT) articles were filed and industry steering committee was formed			Tom Turpen, personal communication
November	Gulf Citrus Growers Association Asian Citrus Psyllid Area Wide Spray Program (2008-2009 Area Wide Dormant Spray) initiated. Spray program for SW FL	Program co-sponsored by UF/IFAS SWFREC and DPI; Activities include: pre-spray psyllid surveys, aerial and ground spraying, and post-spray surveys. Aerial applications (77,000 acres) and ground applications were done from December 2008 to January 2009; Sprays planned for Nov-Dec 2009 and Jan-mid Feb 2010	According to P. Stansly, the program was generally successful and the greatest failure of the program was the inability to include abandoned groves (10,000 acres) in areas managed by SFWMD (Southwest FL Water Management District)	http://www.doacs.state.fl.us/pi/chrp/documents/chrp_update_mar_2009.pdf Phil Stansly, personal communication
December	International Research Conference on HLB	Organized by DPI, ARS, APHIS, UF-IFAS; Sponsored by FL Citrus Mutual, TX Citrus Producers Board, FL Specialty Crop Foundation, Cutrale, FCPRAC, and Sunkist	This was attended by researchers and others from about 25 countries with 425 participants	http://fl-dpi.com/hlb_conference/index.html T. Gottwald, personal communication
December 2008 to March 2009	FCATP negotiated contracts with PIs		For 2008-2009, FCPRAC funded 74 HLB projects reviewed and recommended by NRC; 18 HLB projects reviewed but not recommended by the NRC and 2 HLB projects not reviewed by the NRC; Total Number of HLB projects funded: 94; Total number of funded projects on canker/others:	http://www.flcitrusmutual.com/files/3300e160-1030-4672-a.pdf

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
			12	
2009	Approximately $18 million for HLB/canker research was approved by FCPRAC	Funded by DOC/FCPRAC		Peter McClure, personal communication
January	Area Wide Control of ACP TWG convened	The meeting was organized by APHIS	This TWG met to identify a logical set of key elements related to area-wide control (AWC) of ACP, and assign work to identified groups within the TWG. (Taken from final report)	http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/twg/Psyllid%20Area%20Wide%20Control2.09.09.pdf
January	Indian River Citrus Seminar (Ft. Pierce, FL)	Presented by Florida Grower magazine, the Indian River Citrus League and UF-IFAS	The program included HLB International Perspectives and Research Initiative and Grove Management Practices (Emphasis on Psyllid Management). Drew more than 1,200 attendees and 80 exhibitors	http://www.growingproduce.com/events/flgevents/pdf/IRCS09PromoBrochure_small.pdf
January	Highlands Citrus Grower Forum (Sebring, FL)	UF-IFAS Extension	The purpose of the Forum is to allow growers to gather together and share information about their psyllid and greening management programs, both successes and failures	http://flcitrus.ifas.ufl.edu/Newsletters/Hurner/12%2008%20Dec%20Newsletter%20final.pdf
January	Psyllid Management (Sebring, FL)	UF-IFAS	ACP Management Considerations; Scouting and Monitoring of Psyllids and Predators for Psyllid Management; Biological and Low Volume Sprays to Manage ACP	http://flcitrus.ifas.ufl.edu/Newsletters/Hurner/12%2008%20Dec%20Newsletter%20final.pdf
January	Area Wide Psyllid Control	Coordinated by Story Citrus Services, Lake Wales, FL (Polk County, central FL)	Participated in by 16 companies; 2,300 acres covered	http://southeastagnet.com/category/citrus/grower-trials/page/2/

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
January & February	Citrus Production School (Arcadia, FL)	UF-IFAS Extension	Topics discussed: Citrus Canker—factors and practices to consider about the disease; Developing programs to assure disease-free citrus nursery trees; Citrus greening—understanding this new disease and its identification in field situations; Controlling citrus psyllid—a vector of citrus greening; Understanding what happens in an agricultural use inspection by the Fla. Department of Agriculture; Citrus BMP cost share opportunities	http://desoto.ifas.ufl.edu/pdf/Citrus%20Newsletters/2006%20Newsletters/January%202006%20No%20Sig%20%20with%20Banner%20PDF.pdf
February	NPDN SOP for Citrus Greening and Asian Citrus Psyllid was updated	Version 2.0 was made available to all NPDN diagnosticians	NPDN diagnosticians were provided with proper protocols and training to screen for citrus greening and the Asian citrus psyllid. NPDN labs are able to serve as sources for sample overflow when the need for citrus greening sampling arises. NPDN labs are familiar with proper communication protocols involving the USDA-APHIS-PPQ and their local state department of agriculture.	Amanda Hodges, personal communication
February	A presentation on Citrus Nutrition and Psyllid Management was given by UF-IFAS		The presentation focused on nutrition as it relates to management of HLB and suggestions to lower citrus nutrition costs through adjusting rates and VRT application technology	http://flcitrus.ifas.ufl.edu/Newsletters/Hurner/12%2008%20Dec%20Newsletter%20final.pdf
February	Technical Working Group Recommendations on "Area Wide Psyllid Control" was released	APHIS		http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/twg/Psyllid%20Area%20Wide%20Control2.09.09.pdf

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
February	Advanced Citrus Technologies Corporation (ACT) structure was put on hold to pursue an alternative non-profit corporate structure, a University of Florida Direct Support Organization (DSO)	Direct Service Organization (DSO) formation requested by Senator J.D. Alexander		Tom Turpen, personal communication
March	UF Trustees certify the Citrus Research and Development Foundation (CRDF); CRDF Articles were filed		CRDF's role: Assist in the coordination of solicitation of funding from all sources, manage research projects, develop and commercialize research results into products, manage IP rights, anticipate and navigate regulatory obstacles	Tom Turpen, personal communication
April	Citrus Greening Symposium (2nd)	Conducted by UF/IFAS Extension, FCPRAC, and the Greening Research Task Force	To discuss production systems, plant improvement, vector management, horticultural responses to HLB and disease detection and spread	http://stlucie.ifas.ufl.edu/pdfs/citrus/2009%20Institute%20Brochure.pdf
April	HLB Database created	The Citrus Greening Database is a cooperative effort between UF/IFAS and the Florida Center for Library Automation (FCLA).	To compile and centralize worldwide information related to HLB in a user friendly database that can be easily accessed by everyone. URL: http://swfrec.ifas.ufl.edu/hlb/database/index.htm	http://swfrec.ifas.ufl.edu/hlb/database/
April	Citrus Canker and Greening Field Day was held at the SWFREC		The objective of this field day is to inform growers about the work with citrus canker and greening underway at the SWFREC in Immokalee	http://www.imok.ufl.edu/events/field_days/SWFREC_canker_greening_fd_0409.pdf

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
April	UF/IFAS Citrus Canker and Greening Seminar held at the SWFREC	More than eighty citrus growers, industry representatives, and other clientele participated in this event	The following presentations were given: "Role of Scouting in the Integrated Management of ACP, "Economic Analysis of HLB Foliar Management with the Application of Nutritionals and SARs" and "Progression of Greening Symptoms and Nutrient/SAR Supplements to Mitigate Greening Symptoms"	http://www.imok.ufl.edu/newsletter/su_vol5no1.pdf
April 22	Congressman Adam Putnam (R-Fla) told a US House panel that the federal government must dramatically increase the amount of funding it is providing if it is to save America's citrus crop from a disease known as "citrus greening."	Putnam spoke on behalf of 27 members of Congress who have signed a letter calling for the Appropriations subcommittee on agriculture to add $64 million to the effort to expand methods of diagnosing and controlling the disease		http://southeastagnet.com/category/citrus/grower-trials/page/2/
May	Initial board meeting of CRDF was held	Tom Turpen agrees to serve as Interim COO		Tom Turpen, personal communication
June	Educational Sessions Agenda: HLB Research Program—Today and Tomorrow	Organized by the FL Citrus Mutual; approx 700 people registered for this event	To provide an overview of the current research program and an introduction into all the work that will have to be done downstream from the discovery research before it actually benefits growers, with Dr. Scorza (USDA-ARS) giving a real-world example of getting his plum through the regulatory maze	Peter McClure, personal communication

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
June	DPI was requested to coordinate a pilot aerial spray program for psyllid and Caribbean fruit fly control in West St. Lucie and Indian River Counties, FL	Request made by Indian River Citrus League Director (Doug Bournique)		Peter McClure, personal communication
June-July	FCRICC, FCPRAC, CRDF reps visit USDA and the US Environmental Protection Agency (EPA)		USDA (Gail Wisler, National Program Leader, Horticulture and Sugar) agreed to send low volume aerial application experts from Texas A&M to FL to review and assist in the development of low volume technology for psyllid control	Peter McClure, personal communication
July	A meeting regarding a regional pilot program for psyllid and Caribbean Fruit Fly control was held.	Organized by the Indian River Citrus League	Attendees at the meeting indicated their agreement to an aerial spray program as soon as possible; Participation and role of DPI was determined	Peter McClure, personal communication
August 19-20	Citrus Expo 2009 held in Ft. Myers, FL	This is a self-sustaining event, organized by the Southeast AgNet/Citrus Industry Magazine, with solicited input from growers, researchers, and industry organizations	Since the first meeting in 1992, the Citrus Expo℠ has become the world's premier seminar and trade show program for citrus growers and industry professionals. The primary goal of the expo is to provide education and industry fellowship opportunities annually for growers, industry leaders, decision-makers and vendors.	http://www.citrusexpo.net/index.html
August 19	FCPRAC meeting—held at Citrus Expo 2009	At FCPRAC's direction, Tom Turpen (CRDF) initiated discussions with both private companies and public institutions to accelerate product development partnerships based on the results of prior research awards	One aim is to commercialize a newly discovered active ingredient, a DMDS-based product for psyllid control. Dimethyl disulfide (DMDS), is a compound that has been shown to repel ACP, and was discovered at UF through research funded by the FCPRAC.	Peter McClure and Tom Turpen, personal communication

Date	Event/Activity	Remarks	Purpose/Impact/Result	Reference/Links
August	FCPRAC meeting—held at Citrus Expo 2009	The RFP is posted at the FCPRAC web page and the advertisement will appear in Science magazine for 4 weeks	To solicit proposals that focus on psyllid control and/or that will utilize the *C*Las genomic sequence information recently completed through research funded by the FCPRAC.	Peter McClure and Tom Turpen, personal communication

Appendix J

Funded Projects on Citrus Greening (2008–2010)[a]

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Consequences of HLB infection				
Baldwin, E.	USDA-ARS USHRL	FCPRAC	Effects of HLB on quality of orange juice and identification of HLB-induced chemical signatures in fruit juice and leaves	$147,567
Burns, J. K.; Danyluk, M; Spann, T.; Rouseff, R; Liao, H; Wang, N.	UF-CREC	NIFA (CSREES)	Towards rebalancing sweet orange juice quality in fruit of HLB-infected trees: a transcriptomic and metabolic approach	
Cancalon, P.	UF-CREC	Florida Growers	Determine the damages in orange and grapefruit juice from HLB infected trees	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Dandekar, A.	University of California, Davis Plant Sciences Department	CRB, UC Discovery Grants Program, CWB, UC-CDFA Pierce's Disease Program	Define disease and healthy states using genomics based information	N/A
Muraro, R.	UF-IFAS	FCPRAC	An Economic Model to Evaluate Emerging Solutions to Citrus Greening	
Muraro, R.	UF-IFAS	FCPRAC	Economic Evaluation of Alternate Replanting Production Systems	$50,000
Muraro, R.P. ; Spreen, T. H., Roka, F.M.	UF-CREC	FCPRAC	Economic Impact of Citrus Greening and New Diseases on Florida's Citrus Industry	$210,000
Spreen, T.	UF-IFAS	FCPRAC	Long-Run Processed Orange Production and Price Impacts Associated with Citrus Greening in Florida and São Paulo, Brazil with Implications for Structural Change in the Florida Citrus Sector	$51,110
***C*Las culture, genomics, molecular biology, virulence mechanisms and Koch's postulates**				
Brlansky, R.; Rogers, M.; Davis, M.		NIFA (CSREES)	Development of methods to innoculate plants and psyllids with *Candidatus* Liberibacter asiaticus for infectivity	
Brown, J.	University of Arizona	FCPRAC	The citrus psyllid transcriptome and time course differential gene expression in *Ca.* Liberibacter-infected/free whole psyllids and organs	$165,000

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Chen, J.	USDA ARS San Joaquin Valley Ag Sciences	CRB	Fingerprinting of HLB Strains	N/A
Chen, J.	USDA ARS San Joaquin Valley Ag Sciences	CRB	Fingerprinting of HLB Strains	N/A
Chen, J.	USDA ARS San Joaquin Valley Ag Sciences	FCPRAC	Genetic diversity and fingerprinting of *Candidatus* Liberibacter asiaticus strains	N/A
Chen, J.	USDA ARS San Joaquin Valley Ag Sciences	FCPRAC	Genetic diversity and fingerprinting of *Candidatus* Liberibacter asiaticus strains	N/A
Damsteegt, V.	USDA-ARS NAA FDWSRU	USDA-ARS	Identification, characterization, and biology of foreign and emerging insect-transmitted plant pathogens	N/A
Damsteegt, V.	USDA-ARS NAA FDWSRU	USDA ARS	Identification, Characterization, and Biology of Foreign and Emerging Insect-Transmitted Plant Pathogens	N/A
Damsteegt, V.	USDA-ARS NAA FDWSRU	FCPRAC	Psyllid mediated completion of pathogenicity tests (Koch's Postulates) with a pure culture of the associated Huanglongbing causal bacterium	$175,000
Dawson, W.	UF-CREC	FCPRAC	Sequencing of the HLB genome	
Dollet, M.	CIRAD	FCPRAC	Attempts to in vitro culture *Candidatus* Liberibacter asiaticus isolates in order to fulfill Koch’s postulates	$95,313

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Duan, Y.	USDA-ARS USHRL	FCPRAC	Culturing *Candidatus* Liberibacter asiaticus in vitro and verification if the bacterium is the causal agent of citrus huanglongbing	N/A
Duan, Y.P.	USDA-ARS USHRL	FCPRAC	Dissecting the Disease Complex of Citrus Huanglongbing in Florida	$381,222
Gabriel, D.	UF	USDA-APHIS	Genomic Sequencing of a Curated Florida Citrus Greening Strain of Las	$117,805
Gabriel, D.	UF-IFAS	FCPRAC	Genomic sequencing to closure of a curated Florida citrus greening strain of *Candidatus* Liberibacter asiaticus	$246,601
Hartung, J.	USDA-ARS Beltsville	USDA-ARS	Exotic pathogens of citrus	N/A
Lin, H.	USDA	FCPRAC	Development of SSR markers for detection, genotyping, phenotyping and genetic diversity assessment of *Ca.* Liberibacter strains in Florida	$105,342
Lindeberg, M.	Cornell University	FCPRAC	Bioinformatic characterization and development of a central genome resources website for *Ca.* Liberibacter asiaticus	$140,084
Marrs, B.	Athena Biotechnologies Inc.	FCPRAC	Determining the Microbiome of Healthy and Infected Citrus Phloem Tissue, & Cultivation of *Ca.* Liberibacter	$364,201
Marrs, B.	Athena	CITF,GR	Enzyme Enhanced Cultivation™ to Grow Asian HLB	

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Pietersen, G.	University of Pretoria	N/A	Determine genome variability of Liberibacter africanus	N/A
Schaad, N.	USDA-ARS Ft. Detrick	FCPRAC	Cultivation and Identification of the Causal Agent of Huanglongbing Disease of Citrus	$227,000
Schaad, N.	USDA-ARS Ft. Detrick	CRB	Cultivation and Sequencing of HLB	N/A
Schaad, N.	USDA-ARS Ft. Detrick	USDA-ARS	Identification, Characterization, and Detection of Foreign and Newly Emerging Domestic Bacteria	N/A
Vahling, C.	USDA-ARS USHRL	N/A	Functional genomics of Las	N/A
Wang, N.	UF-IFAS	FCPRAC	Genome sequencing of *Candidatus* Liberibacter asiaticus	$160,916
Wang, N. Dewdney, M; Burns, J.	UF-CREC	NIFA (CSREES)	Characterization of virulence mechanisms of *Candidatus* Liberibacter asiaticus by identification of virulence	
Zhang, S.	UF	N/A	The pathogenicity genes in Las	N/A
Zhou, L.	USDA-ARS USHRL	N/A	Genome project of HLB associate *Ca*.. Liberibacter asiaticus (Las)	N/A

Citrus response to infection: symptoms, defense and susceptibility, *C*Las spread in the plant, systemic acquired resistance

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Albrigo, G.	UF-IFAS	FCPRAC	Characterize the roles of callose and phloem proteins in citrus Huanglongbing (HLB) symptom development	$125,000
Albrigo, G.	USDA-ARS USHRL	USDA, FCPRAC	Detect phloem sieve element plugging materials	N/A
Bowman, K.	USDA-ARS USHRL	FCPRAC	Seed Transmission of HLB	N/A
Brown, L.	Plant Epidemiology and Risk Analysis Laboratory	N/A	Citrus nursery stock as a pathway of introduction for citrus canker, HLB and Asian citrus psyllid from Florida to other citrus producing states in the United States	N/A
Burns, J.	UF-IFAS	FCPRAC	Combating symptom development in fruit from Huanglongbing-infected citrus trees: A transcriptomic, proteomic and metabolomic approach	$179,153
Burns, J. K.; Archer, D. L.; Dusky, J. A.	UF-CREC	NIFA (CSREES)	Infection Processes Associated with Citrus Canker and Citrus Greening	$1,134,593
Dawson, W.	UF-CREC	FCPRAC	Examination of multiplication, movement, distribution, and pathogenicity of HLB and its interaction with CTV in different citrus varieties and relatives	
Garnsey, S.	UF-IFAS	N/A	Define variation in symptom expression in different cultivars and correlation	N/A
Gottwald, T.	USDA-ARS USHRL	N/A	Bacterial survival under field conditions	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Gottwald, T.	USDA-ARS USHRL	FCPRAC	Determination of within tree distribution of Liberibacter sp.	N/A
Graham, J.	UF-IFAS	FCPRAC	Does systemic acquired resistance control HLB disease development?	$66,861
Kang, B.H.	UF-IFAS	FCPRAC	Correlative microscopic and molecular characterization of the microbiome in the citrus phloem tissue	$109,211
Khalaf, A.	UF	CREC	Study host response mechanism(s)	N/A
Lu, H.	UMBC	FCPRAC	Manipulating SA-mediated defense signaling to stimulate systemic acquired resistance to HLB and other pathogens in citrus	$160,897
Moore, G.		NIFA (CSREES)	Is the putative HLB tolerance of some citrus relatives due to an active response?	
Stansly, P.	UF-IFAS	FCPRAC	Evaluation of Systemic Acquired Resistance Inducers Combined with Psyllid Control to Manage Greening in Infected groves.	$50,000
Wang, N.	UF-CREC	FCPRAC	Characterization of the effect of HLB on citrus phloem and phloem transportation	N/A
Wang, N.	UF-CREC	FCPRAC	Characterization the virulence mechanism of the citrus Huanglongbing pathogen *Candidatus* Liberibacter asiaticus	$160,000

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Wang, N.	UF-CREC	FCPRAC	Characterize the microbiomes associated with *Candidatus* Liberibacter asiaticus infected citrus, psyllid, dodder, and periwinkle	$145,114
Wang, N.	UF-CREC	USDA, FCPRAC	Detect phloem sieve element plugging materials	N/A
HLB pathogen and disease detection				
Albrigo, G.	USDA-ARS USHRL	N/A	Remote sensing to identify HLB affected trees	N/A
Cary, R.	Los Alamos National Laboratory	CRB	Lateral Flow Microarray Detection System	N/A
CCRB	CCRB	GR, Other	Identification of *Candidatus* Liberibacter-induced Small RNAs For Early Diagnosis of HLB Citrus Greening	
CCRB			Novel Immunocapture Technology for Field Deployable Nucleic Acid-based Detection of Plant Pathogens	
Costa, N.	INTA-EEA Concordia	INTA	Identification and characterization of harmful organisms-Control HLB	N/A
Damsteegt, V.	USDA-ARS NAA FDWSRU	USDA-ARS	Identification, Characterization, and Detection of Foreign and Newly Emerging Domestic Bacteria	
Dandekar, A.	UC, Davis	CRB	DMS VOC Sensor for Citrus	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Dandekar, A.	UC, Davis	FCPRAC	Identification and modeling of early responses to HLB infection to improve disease management	$298,538
Dandekar, A.	UC, Davis	FCPRAC	A Generalized Reagentless Sensor to Detect Citrus Plant and Fruit Responses	$120,000
Das, A.	National Research Centre for Citrus	ICAR, Ministry of Agriculture, India	Development of rapid diagnostics (PCR based)	N/A
Davis, R.	UF-IFAS	CITF, GR	Development of Light Microscope to Detect and Monitor HLB	
Davis, R.	Molecular Plant Pathology Laboratory	USDA-ARS	Genome-based detection, ID, and classification	N/A
Dawson, W.	UF-CREC	FCPRAC	Development of simple, sensitive, and rapid diagnostic procedures for large scale detection of the citrus greening pathogen	N/A
Ehsani, R.	UF-CREC	FCPRAC	Detecting Citrus Greening (HLB) Using Multiple Sensors and Sensor Fusion Approach	$290,000
Ehsani, R.	UF-CREC	FCPRAC, NASA	Remote sensing to identify HLB affected trees	N/A
Ehsani, R.	UF-CREC	DOC, GR	Early Detection and Mapping of (HLB) Using Ground-Based and /or Aerial Hyperspectral or Other Imaging	

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Filho, A.	CACSM	FCPRAC	Diagnosis of *Candidatus* Liberibacter asiaticus in plant and vector based on molecular and serological approaches.	$32,500
Filho, F.	Agdia, Inc.	N/A	Preparation of antibodies against *Candidatus* Liberibacter asiaticus	N/A
Gottwald, T.	USDA-ARS USHRL	FCPRAC	Disease detection via hyperspectral imaging	N/A
Gottwald, T.	USDA-ARS USHRL	FCPRAC	Evaluation of detection protocols	N/A
Gottwald, T.	USDA-ARS USHRL	CPHST (CHRP)	Spectroscopic studies of Citrus Huanglongbing Detection	N/A
Gowda, S.	UF-IFAS	FCPRAC	Development of sensitive, non-radioactive and rapid tissue blot diagnostic method for large-scale detection of citrus greening pathogen	$41,040
Hartung, J.	USDA-ARS Beltsville	FCPRAC	Preparation of antibodies against *Candidatus* Liberibacter asiaticus	$221,900
Irey, M.	US Sugar	FCPRAC	Support for the Southern Gardens Diagnostic Laboratory	$226,444
Lee, R.	USDA ARS National Clonal Germplasm Repository	N/A	Improving The Prospects for Detection and Eradication of Citrus Huanglongbing	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Levy, L.	USDA APHIS PPQ CPHST	USDA-APHIS	Development of CANARY B-Cell Sensing Technology for Rapid, Sensitive Detection of Plant Pathogens and Evaluation of PCR Sample-prep Cartridges for use in Trace-level Detection of Plant Diseases	N/A
Levy, L.	USDA APHIS PPQ CPHST	USDA-APHIS	Real-time PCR for Diagnostic Detection of Citrus Greening or HLB from plant samples (AMS Sample processing)	N/A
Li, W.	USDA APHIS PPQ CPHST NPGBL	N/A	Detection methods of citrus pathogens	N/A
Li, W.	National Plant Germplasm and Biotechnology Laboratory	N/A	Development of a real-time PCR for detection of citrus greening disease	N/A
Li, W.	USDA APHIS PPQ CPHST	USDA-APHIS	Sampling Methods for Improved Detection and Identification of the Causal Bacterium of citrus HLB	N/A
Li, W.	USDA APHIS PPQ CPHST	CPHST (CHRP)	Sampling Methods for Improved Detection and Identification of the Causal Bacterium of citrus HLB	N/A
Liu, Z.	National Plant Germplasm and Biotechnology Laboratory	N/A	DNA Chip for Rapid Detection of Regulated Plant Pathogens	N/A
Moore, G.	UF-IFAS	FCPRAC	Gene Expression in HLB Infected Citrus Trees	$60,000

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Reyes, J. De Corcuera	UF-CREC	FCPRAC	Identification of metabolite changes in citrus leaves induced by Huanglongbing disease. A first step towards developing a rapid method for early detection of the disease	$33,150
Roberts, P.	UF-IFAS	FCPRAC	Diagnostic Services for growers for detection of HLB to aid in management decisions	$187,601
Salas, B.	USDA APHIS PPQ CPHST	USDA-APHIS	Sentinel Tree Monitoring for Citrus Canker and HLB in Texas	N/A
Schumann, A.	UF-CREC	FCPRAC	Determine if HLB can be detected earlier, and if symptoms can be distinguished from other leaf discolorations (eg., mineral nutrient deficiency) using machine vision	$50,000
Spann, T.	UF-CREC	FCPRAC	Improving micro-element management for easier detection of HLB symptoms	N/A
Spann, T.	UF-CREC	FCPRAC	Using physical and chemical property changes of citrus leaves as early indicators of HLB infection and effects of added plant nutrients	$164,631
Su, H.J.	Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan	Department	Detection and health management of HLB	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Triplett, E.	UF-IFAS	FCPRAC	Integrative Approaches to Discover Pathogenesis-Associated Proteins from the Causal Agent of Citrus Greening Disease and Build New Diagnostic Tools	$400,000
HLB epidemiology and mitigation of HLB by cultural practices or competing microbes				
Albrigo, G.	USDA-ARS USHRL	FFVA, FCPRAC	Kill abandoned and HLB affected trees without removal	N/A
Bar-Joseph, M.	Retired	N/A	Developing a comprehensive working plan to deal with HLB infections in new citrus growing areas	N/A
Barkley, P.	USDA-ARS USHRL	Australian citrus industry, and Commonwealth Government	Preparedness for an incursion of HLB and/or its vectors	N/A
Bartels, D.	USDA APHIS PPQ CPHST	USDA-APHIS	Area-Wide Management of Asian Citrus Psyllid (ACP), *Diaphorina citri* in Texas	$116,500
Bassanezi, R.	Fundecitrus	FCPRAC	Comparative epidemiology of citrus huanglongbing (greening) caused by *Candidatus* Liberibacter asiaticus and *Ca*. L. americanus	$83,825
Bassanezi, R.	Fundecitrus	FCPRAC	Reduction of bacterial inoculum and vector control as strategies to management of citrus huanglongbing (greening)	$49,000
Bassanezi, R.	USDA-ARS USHRL	Fundecitrus, FAPESP	Study the effect of HLB control strategies on HLB spatio-temporal progress	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Beattie, A.	U Western Sydney and South China Ag Univ., Guangzhou, funded by Australian Dept. of Innovation, Industry, Science and Research	N/A	Developing Ecologically Sustainable Pest and Disease Management Strategies for Citrus, with Emphasis on Huanglongbing: 1) impacts of canopy and root temperatures *C*Las incidence 2) impacts of host and leaf age on severity of HLB and ACP feeding behavior 3/4) impacts of azadirachtin and guava volatiles on ACP feeding and oviposition 5) symptoms in co-infections of CTV and HLB 6) iodine-starch test for validating HLB symptoms	N/A
Bowman, K.	USDA-ARS USHRL	FCPRAC	High density plantings as a strategy for increasing profitability of citrus exposed to HLB	N/A
Brlansky, R.	UF-IFAS	FCPRAC	Alternative Hosts of HLB to Assist in Disease Management	$205,000
Castle, W.	UF-CREC	N/A	Can windbreaks be designed to aid in HLB/psyllid management	N/A
Filho, A.	Universidade de São Paulo	FAPESP, USDA, Fundecitrus	Epidemiology and Management of HLB	N/A
Filho, A.	Universidade de São Paulo	USDA. Fundecitrus, Fapesp	Epidemiology and Management of HLB	N/A
Filho, A.	Universidade de São Paulo	FAPESP, USDA, Fundecitrus	Relative importance of HLB control measures	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Fowler, L.	Plant Epidemiology and Risk Analysis Laboratory	Funded	Assessing Host Range of Citrus Greening—*Candidatus* Liberibacter—all strains	N/A
Futch, S.	UF-CREC	FCPRAC	Treatment of citrus stumps with herbicides to minimize sprout formation	$16,000
Gottwald, T.	USDA-ARS USHRL	FCPRAC, USDA/APHIS	Disease control via epidemic management	N/A
Gottwald, T.	USDA-ARS USHRL	USDA-ARS	Domestic, Exotic, and Emerging Diseases of Citrus, Vegetables, and Ornamentals (Deed)	N/A
Gottwald, T.	USDA-ARS USHRL	FCPRAC	Epidemiology and disease control of HLB	$253,251
Gottwald, T.	USDA-ARS USHRL	FCPRAC	Infected fruit peels as sources of inoculum (passive dispersal)	N/A
Gottwald, T.	USDA-ARS USHRL	FCPRAC	Mechanical transmission from infected fruit from packinghouse as sources of infection	N/A
Gottwald, T.; Gilligan, C.	USDA-ARS & University of Cambridge	USDA-APHIS	Disease Modeling via Stochastic Simulation to Test Disease Control/Mitigation Strategies and Maximize Regulatory Intervention	N/A
Graham, J.	UF	FCPRAC	Transmission of HLB by citrus seed	$36,561

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Pietersen, G.	ARC-Plant Protection Research Institute	Citrus Research International	Epidemiology of citrus greening: Investigation into alternate hosts of Liberibacter africanus	$80,000
Powell, C.; Duan, Y.	UF, Florida; USDA ARS-USHRL	USDA-APHIS	Characterization of Seed Transmission of Citrus Huanglongbing	N/A
Roberts, P.	UF-IFAS	FCPRAC	Spatial and Temporal Incidence of *Ca*. Liberibacter in Citrus and Psyllids detected Using Real-time PCR	$90,120
Roberts, P.	UF-IFAS	NIFA (CSREES)	Assessment of Zanthoxyium fagara as a natural host for citrus greening	
Rouse, R.	UF-IFAS	FCPRAC	Cultural Practices to Prolong Productive Life of an HLB Infected Tree and Evaluation of Systemic Acquired Resistance inducers combined with Psyllid Control to manage Greening	$214,881
Schumann, A.	UF-IFAS	FCPRAC	Intensively managed citrus production systems for early high yields and vegetative flush control in the presence of greening disease	$225,180
Setamou, M.	TAMU	FCPRAC	Coupling citrus flush management and dormant chemical spray as a strategy to control populations of Asian citrus psyllid	$56,794
Singh, M.	UF-CREC	FCPRAC	Elimination of HLB infected trees without physical removal through application of herbicides	$43,909

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Singh, M.	UF-CREC	FFVA, FCPRAC	Kill abandoned and HLB affected trees without removal	N/A
Spann, T.	UF-IFAS	FCPRAC	Strategies to minimize growth flushes of mature citrus trees with pruning practices and plant growth regulators to reduce psyllid feeding	$137,539
Stansly, P.	UF-IFAS	FCPRAC	Creation and Maintenance of an Online Citrus Greening Database	$25,153
Stenger, D.	USDA-ARS Parlier	USDA-ARS	Epidemiology and Management of *Xylella fastidiosa* (Xf) and Other Exotic and Invasive Diseases and Insect Pests	N/A
Wulff, N.A.	Fundo de Defesa da Citricultura (Fundecitrus)	Fundecitrus, FAPESP	Molecular epidemiology	N/A
ACP monitoring and behavior, cultivation and relationship to *C*Las				
Brlansky, R.; Rogers, M.	UF-CREC	USDA-APHIS	Seasonality of the Detection of the Citrus Huanglongbing Bacterium in Field Collected and/or Fed Asian Citrus Psyllids (*Diaphorina citri*) in Florida	$38,500
Brown, J.	Arizona Board of Regents (University of Arizona)	FCPRAC	Gross and fine structure localization of Liberibacter in citrus psyllid *Diaphorina citri* organs: elucidating the transmission pathway	$119,998
Cicero, J.	University of Arizona	FCPRAC	Gross and fine structure localization of Liberibacter in citrus psyllid *Diaphorina citr*i organs: elucidating the transmission pathway	$120,000

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Czokajla, D.	Alpha Scouts, INC.	USDA, NSF, SBIR	Develop/improve monitoring trap	N/A
da Graça, J.	Texas A&M University Kingsville, Citrus Center	CPHST (CHRP)	Detection of Transmissible Citrus HLB in ACP by Bioassay in Susceptible Citrus Seedlings	N/A
da Graça, J.	Texas A&M Kingsville Citrus Center	USDA-APHIS	Detection of Transmissible Citrus HLB in ACP by Bioassay in Susceptible Citrus Seedlings	$11,948
Duan, Y.	USDA-ARS USHRL	FCPRAC	Microbial ecology of citrus huanglongbing and its insect vector, Asian citrus psyllid	
FDACS-DPI	FDACS-DPI	CITF,GR	Measuring Flight Activity of *Diaphorina citri*, the Vector of Citrus Greening Disease and Determining Seasonality of Transmission	
Hall, D.	USDA-ARS USHRL	FCPRAC	Artificial systems for *Diaphorina citri*	N/A
Hall, D.	USDA-ARS USHRL	FCPRAC	Asian Citrus Psyllid—Sampling, Biological Control, and Seasonal Profile of HLB in Adult Psyllids	N/A
Hall, D.	USDA-ARS USHRL	USDA/ARS, Box tax	Assessing psyllid flight activity to and from groves	N/A
Hall, D.	USDA-ARS, USHRL	FCPRAC	Pathogen-Vector Relations between Asian Citrus Psyllid and Liberibacter asiaticus	$112,000

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Hall, D.	USDA-ARS USHRL	FCPRAC	Phenology of Liberibacter in Field Populations of Adult *Diaphorina citri*	N/A
Hert, M.	USDA-ARS USHRL	N/A	Establishment of psyllid cell culture	N/A
Holtz, T.	USDA-APHIS	N/A	*Diaphorina* citri Kuwayama/ Asian citrus psyllid	N/A
Hunter, W.	USDA-ARS USHRL	FCPRAC	Psyllid genomics/metagenomics, immune responses..	N/A
Keyhani, N.	IFAS	FCPRAC	Development of Asian citrus psyllid, *Diaphorina citri,* insect cell lines	$80,000
Lopes, J.	University of São Paulo	FCPRAC	Factors influencing acquisition and inoculation of *Candidatus* Liberibacter spp. by *Diaphorina citri*	$63,200
Lopes, S.	Fundo de Defesa da Citricultura (Fundecitrus)	FAPESP, Applied for grants from Florida Citrus Industries	Determine temperature limits for *Ca*. L. americanus and *Ca*. L. asiaticus multiplication in citrus and D. *citri*	
Mizell, R.	UF-IFAS	FCPRAC	An effective trap for Asian citrus psyllid that can be used to monitor groves and plants for sale	$100,000
Patt, J.	USDA-ARS Subtropical Agricultural Research Center	USDA-ARS	Asian Citrus Psyllid-host plant finding behavior	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Rogers, M.	UF-CREC	FCPRAC	Effects of nutrition and host plant on biology and behavior of the Asian citrus psyllid and implications for managing psyllid populations	$89,586
Rogers, M.	UF-CREC	FCPRAC	Huanglongbing: Understanding the vector-pathogen interaction for disease management	$519,429
Rogers, M.	UF-CREC	FCPRAC	Interaction between *D. citri* and *Ca.* Liberibacter asiaticus	N/A
Rogers, M.; Brlansky, R.	UF-CREC	FCPRAC	Investigation of psyllid transmission of the citrus greening pathogen and methods for preventing pathogen transmission	$95,000
Rogers, M.; Brlansky, R.	UF-CREC	FCPRAC	Pathogen acquisition and transmission by psyllids from symptomatic and asymptomatic trees in the field	$100,000
Stelinski, L.	UF-IFAS	FCPRAC	Quantitative measurement of the movement patterns and dispersal behavior of Asian citrus psyllid in Florida for improved management	$101,631
ACP chemical, biological or biochemical management chemical attractants and repellants				
Aubert, B.	USDA-ARS USHRL	CIRAD-INRA	Collect *Tamarixia radiata* from Reunion/Guadeloupe and other countries	N/A
Avery, P.	USDA-ARS USHRL	N/A	Assessing grower psyllid management strategies	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Avery, P.; Hunter, W.; Hall, D.; Jackson, M.; Powell, C.; Rogers, M.	Submitted to IRCHLB		Investigations of the feasibility for managing the Asian citrus psyllid using Isaria fumosorosea	
Beattie, A.	University of Western Sydney	ACIAR	Huanglongbing management for Indonesia, Vietnam and Australia 1) altitude and natural enemies affects on HLB in Java 2) impacts of guava volatiles the Mekong Delta 3/4) evaluation of mineral oils for reducing HLB in Viet Nam and Indonesia 5) timing of imidacloprid soil drenches 6) plant nutrition and HLB symptoms 7) the systematic status of *Murraya* and Merrillia 8) HLB and phloem degeneration	N/A
Borovsky, D.	UF-IFAS	FCPRAC	Control of the Asian citrus psyllid, *Diaphorina citri* Kuwayama with protease inhibitors and RNAi.	$196,401
Bournique, D.	IRCL	FCPRAC	Validation of Area-Wide Management of Asian Citrus Psyllid	$67,875
Brlansky, R.	UF- IFAS	FCPRAC	Effects of Insecticides on Pathogen Transmission by the Asian Citrus Psyllid	$55,000
Carlson, J.; Ciomperlik, M.	USDA APHIS PPQ CPHST	CPHST Internal Funds	Evaluation of Insecticidal Efficacy on Asian Citrus Psyllid (*Diaphornia citri* Kuwayama) using Foliar and Drench Applications	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Cox, K.	Cornell University	FCPRAC	Management of Pyslla in tree fruit crops using RNA interference	$106,915
Falk, B.	UC Davis	FCPRAC	Controlling HLB by controlling psyllids with RNA interference	$198,274
Flores, D.	USDA APHIS PPQ CPHST	USDA-APHIS	Evaluating the Population Dynamics and Biological Control of the Asian Citrus Psyllid (ACP) in the Rio Grande Valley of Texas	$15,000
Gravena, S.	Gravena Research, Consulting and Training, LTD.	Insecticide companies	Efficacy of biocontrol and insecticides on ACP	N/A
Hall, D.	USDA-ARS USHRL	FCPRAC	Asian Citrus Psyllid—Sampling, Biological Control, and Seasonal Profile of HLB in Adult Psyllids	$81,000
Hall, D.	USDA-ARS USHRL	N/A	Development of Biological Control And IPM Technology For Exotic Insect Pests	N/A
Hall, D.	USDA-ARS USHRL	FCPRAC	Efficacy of Seasonal Insecticide Programs for Suppressing HLB in New Citrus Plantings	$99,701
Hall, D.	USDA-ARS USHRL	USDA-ARS	IPM Technologies for subtropical insect pests	N/A
Hall, D.	USDA-ARS USHRL	FCPRAC	New Biological Control Agents for Asian Citrus Psyllid.	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Hall, D.	USDA-ARS USHRL	Pepsico	Asian Citrus Psyllid Attractants and Repellants	$26,000
Hoy, M.	UF-IFAS	CITF,GR	Improved Control of Psyllid, with Silwet L-77 and Reduced Rates of Insecticides	
Ichinose, K.	Japan International Research Center of Agricultural Sciences	N/A	Population dynamics, efficacy of systemic insecticides and guava	N/A
Kawano, S.	Okinawa Prefectural Agricultural Research Center	N/A	Developing IPM for HLB	N/A
Lapointe, S.	USDA-ARS	FCPRAC	A push-pull strategy for control of the Asian citrus psyllid	$36,879
Lee, R.	USDA ARS National Clonal Germplasm Repository	Citrus Research Board	Huanglongbing: Development of Information Needed for Avoidance/Management	
Lopez-Arroyo, I.	INIFAP	INIFAP	Management of *Diaphorina citri*	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Mangan, R. L.; Adamczyk, J. J.; Thomas, D. B.; Malik, N.; Hallman, G. J.; Fletcher, R.; Fletcher, R.; De Graca, J.; Setamou, M.; Skaria, M.	USDA-ARS USHRL	N/A	Development of an Area-wide Approach for Controlling Infection and Spread of HLB of Asian Citrus Psyllid	N/A
Ohto, Y.	Japan International Research Center for Ag Sciences	N/A	IPM in areas with endemic HLB	N/A
Patt, J.	USDA-ARS-Subtropical Agricultural Research Center, Beneficial Insects Research Unit, Weslaco, TX	USDA ARS, FL 'Box Tax' grant	Characterize the odor emitted by flushing terminal shoots of ACP host plants	N/A
Qureshi, J.	UF	FCPRAC	Sampling Plans to Guide Decision Making for Control of Asian Citrus Psyllid	$200,373
Ramallo, J.	Estocion Experimental Agroindustrial Orisk Volunteer-AFINDA	N/A	Psyllid management	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Ramallo, J.	Estocion Experimental Agroindustrial Orisk Volunteer-AFINDA	N/A	Psyllid management	N/A
Rogers, M.	UF-CREC	FCPRAC	Direct Grower Assistance: Development and evaluation of citrus grower psyllid management programs	$107,723
Rogers, M.	UF-CREC	FCPRAC	Resistance and cross-resistance development potential in Asian citrus psyllid to insecticides and its impact on psyllid management	$140,315
Rogers, M.; Brlansky, R.	UF-CREC	FCPRAC	Effects of insecticides on pathogen transmission by the Asian citrus psyllid	N/A
Rogers, M.	UF-CREC		Development and Evaluation of Citrus Grower Psyllid Management Programs	
Rogers, M.	UF-CREC	FCPRAC	Development of psyllid baseline toxicology information	$65,000
Salyani, M.	UF-CREC	FCPRAC	Evaluation and development of effective ultra low volume spray technologies for management of the Asian citrus psyllid	$99,058
Salyani, M.; Ehsani, R.	UF-CREC	FCPRAC	Spot-treatment of young leaf flushes for chemical control of psyllid	N/A
Salyani, M.; Stelinski, L.	UF-CREC	FCPRAC	Identifying appropriate spray technologies for economical and effective control of citrus psyllid	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Saylani, M.	UF-CREC	FCPRAC	Evaluation and development of effective ultra low volume spray technologies for management of the Asian citrus psyllid	N/A
Schumann, A.	UF-IFAS	FCPRAC	Advanced control system for variable rate application of fertilizer and pesticide to trees in the presence of greening and canker	$29,846
Setamou, M.	Texas A&M University Kingsville, Citrus Center	Texas Citrus Producers Board, USDA APHIS	Development of areawide management strategies of Asian Citrus Psyllid	N/A
Setamou, M.	Texas A&M University Kingsville, Citrus Center	FCPRAC	Coupling citrus flush management and dormant chemical spray as a strategy to control populations of Asian citrus psyllid	$56,794
Stansly, P.	UF-IFAS	FCPRAC	Development and Delivery of Comprehensive Management Plans for Asian Citrus Psyllid Control in Florida Citrus	$136,353
Stansly, P.	UF-IFAS	FCPRAC	Enhanced Biological Control of Asian Citrus Psyllid in Florida Through Introduction and Mass Rearing of Natural Enemies	$153,062
Stansly, P.	UF-IFAS	FCPRAC	Integrated Pest Management of ACP	N/A
Stansly, P.	UF-IFAS	FCPRAC	Ultralow Volume and Aerial Application of Insecticides and Horticultural Mineral Oil to Control Asian Citrus Psyllid in Commercial Orchards	$54,066

Principal Investigator	**Affiliation**	**Funding source**	**Project Title/Research Topic**	**Budget Year One**
Stelinski, L.	UF-CREC	FCPRAC	Development and optimization of biorational tactics for Asian citrus psyllid control and decreasing huanglongbing incidence	$92,778
Stelinski, L.	UF-CREC	FCPRAC	Development of attractants for *Tamarixia radiata*, a parasitoid of Asian Citrus Psyllid (ACP), for improved biological control of ACP	$43,098
Stelinski, L.	UF-CREC	FCPRAC	Development of Effective Guava-based Repellent to Control Asian Citrus Psyllid and Mitigate Huanglongbing Disease Incidence	$159,869
Stelinski, L.	UF-CREC	FCPRAC	Identification of psyllid attractants and development of highly effective trapping and attract-and-kill methods for improved psyllid control	$180,049
Stelinski, L.	UF-CREC	FCPRAC	Development of attractants for Asian citrus psyllid	$155,000
Taylor, E.C.	CECNEA-Concordia-Argentina	Government	Control of population and movements of *D. citri*	N/A
Thomas, D.	USDA-ARS	USDA Specialty Crops Grant	Development of areawide control system of ACP	N/A
Waddill, C.	UF-IFAS		Management of Greening at SWFREC and in Commercial Groves in SW Florida	
Waddill, C.	UF-IFAS	DOC,GR	Management of Greening at SWFREC and in Commercial Groves in SW Florida	

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Woods, D.	Inscent Irvine, Inc.	CRB	Attractants and Repellants for ACP	N/A
Yamamoto, P.	Fundecitrus	FCPRAC	Can insecticides and mineral oil avoid transmission of *Candidatus* Liberibacter asiaticus by *Diaphorina citri?*	$39,275
Yamamoto, P.	Fundo de Defesa da Citricultura (Fundecitrus)	Papesp	Influence of systemical/contact insecticides, and mineral oil on Ca. Liberibacter transmission	N/A
ACP trapping and repelling plants				
Gmitter, F.	UF-IFAS	FCPRAC	Development of Transformation Techniques for *Murraya*, to Engineer a Deadly Trap Plant	$35,000
Gottwald, T.	USDA-ARS USHRL	FCPRAC	Can guava plantings be used to protect citrus nursery production facilities?	N/A
Gottwald, T.	USDA-ARS USHRL	FCPRAC	Efficacy of interplanting citrus with guava as a control strategy for Huanglongbing	$240,193
Gottwald, T.	USDA-ARS USHRL	FCPRAC	SAGE (Southeast Asia Guava Effect) efficacy of interplanting guava, *M. paniculata*, and other plant species with citrus as control strategies for Huanglongbing in Florida commercial citrus groves	N/A
Hall, D.	USDA-ARS USHRL	FCPRAC	Effects of interplanting citrus with guava on infestations by citrus aphids, leafminers, mites and other insect pests	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Salinas, E.	USDA APHIS PPQ	USDA-APHIS	Huanglongbing (Citrus Greening) and ACP Management by Interplanting Guava with Dense Planted Citrus	$20,000
Citrus genomics and transcriptomics				
Gmitter, F.	UF-CREC	FCPRAC	International Citrus Genome Consortium (ICGC): Providing Tools to Address HLB and Other Challenges	$1,300,000
Kang, B-H	UF, Department of Microbiology and Cell Science	FCPRAC	Correlative microscopic and molecular characterization of the microbiome in the citrus phloem tissue	$109,211
Machado, M.	CACSM	FCPRAC	Analysis of transcriptome of citrus infected with *Ca.* Liberibacter asiaticus and *Ca.* L. americanus	$82,000
Conventional citrus breeding for resistance				
Bowman, K.	USDA-ARS USHRL	FCPRAC	Development of Promising New Rootstocks and Scions for Florida Citrus	$150,000
Bowman, K.	USDA-ARS USHRL	USDA-ARS	Genetic improvement of citrus	N/A
Dawson, W.	UF-IFAS	FCPRAC	Examine the response of different genotypes of citrus to citrus greening (Huanglongbing) under different conditions	$226,391
Filimonova, S.	UF-CREC	FCPRAC	Rutaceous Germplasm Preservation	$71,000
Folimonova, S	UF-CREC	NIFA (CSREES)	Characterization of tolerance to HLB	

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Gmitter, F.	UF-CREC	FCPRAC	Assessment of the HLB Resistance and Tolerance in Citrus and Its Relatives	$50,000
Gmitter, F.	UF-CREC	FCPRAC, NVDMC	Genetic resistance to disease: native and transgenic resistance	N/A
Gmitter, F.	UF-CREC	FCPRAC	Identification and Characterization of HLB Survivors	$76,312
Gmitter, F.	UF-CREC	FCPRAC	Surviving HLB and Canker: Genetic Strategies for Improved Scion and Rootstock Varieties	$840,046
Gottwald, T.	USDA-ARS USHRL	N/A	Transmission methods for screening germplasm: Exposing germplasm to psyllids under grove conditions	N/A
Grosser, J.	UF-CREC	FCPRAC	Development of HLB-Resistant Citrus	N/A
Lee, R.	USDA-ARS UC Riverside	USDA-ARS	Management of Citrus and Date Genetic Resources and Information	N/A
Lee, R.	USDA	FCPRAC	Recovery of Citrus Germplasm in Florida	$36,970
Machado, M.	Centro de Citricultura Sylvid Moreira	FAPESP, CNPq, EMBRAPA	Survey for tolerance/resistance in germplasm collection	N/A
Moore, G.	UF-IFAS	FCPRAC	Evaluate Differences in Response to HLB by Scions on Different Rootstocks	$55,000
Roose, M.	UC Riverside	CRB	Citrus Rootstock Evaluation	N/A

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Roose, M.	UC Riverside	CRB	New Citrus Breeding	N/A
Stover, E.	USDA-ARS USHRL	USDA/ARS, FCPRAC	Development of HLB and Canker Resistant Citrus	N/A
Stuchi, E.S.	Embrapa Cassava and Tropical Fruits Citrus Experimental Station, Bebedouro, SP, Brazil	Embrapa, Fapesp, CNPq, Coopercitrus (Grower's cooperatives), CREDIATRUS	Selection of resistant/tolerant cultivars to CVC, CDS and HLB (start if funding)	N/A
Transgenic and viral/bacterial vector mediation of citrus resistance to HLB				
Dawson, W.	UF-IFAS	FCPRAC	Identify and deliver antibacterial peptides and/or proteins for the control of citrus greening (Huanglongbing or HLB)	$309,194
Dawson, W.	UF-CREC)	FCPRAC	Increase the capacity of the Core Citrus Transformation Laboratory	N/A
Grosser, J.	UF-IFAS	FCPRAC	Accelerating the Commercialization of Transformed Juvenile Citrus	$178,000
Grosser, J.	CREC-UF	FCPRAC	Increasing the Capacity of the University of Florida's CREC Core Citrus Transformation Facility (CCTF)	$85,000
Gurley, W.	UF-IFAS	FCPRAC	Engineering citrus for resistance to Liberibacter and other phloem pathogens	$112,894

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Gurley, W.	UF-IFAS	NIFA (CSREES)	Genetic modification of citrus resistance against citrus greening: preventing expression of anti-bacterial peptides	
Hailing, J.	UC Riverside	CRB	Small RNA for HLB Plant Response	N/A
Moore, G.	UF-IFAS	FCPRAC	Agrobacterium-mediated Genetic Transformation of Mature Citrus Tissue	$125,000
Moore, G.	UF-IFAS	FCPRAC, USDA NRI Specialty Grants	Expression of pathogen-response genes in citrus	N/A
Pena, L.	IVIA	FCPRAC	Development of transformation systems for mature tissue of Florida commercial varieties, and strategies to improve tree management	$145,351
Reddy, J.; Gabriel, D.	Integrated Plant Gentics, Inc.	FCPRAC	Development of Transgenic Valencia and Hamlin Orange for HLB resistance	N/A
Reddy, J.; Gabriel, D.	Integrated Plant Genetics, Inc.	USDA-APHIS	Integrated Plant Genetics (IPG) Gabriel Transgenic Project	$128,000
Song, W.	UF-IFAS	FCPRAC	Engineering Resistance Against Citrus Canker and Greening Using Candidate Genes	$115,213
Stover, E.	USDA-ARS USHRL	FCPRAC	A secure site for testing transgenic and conventional citrus for HLB and psyllid resistance	$93,049
Stover, E.	USDA-ARS USHRL	FCPRAC	Production of Transgenic Commercial Cultivars Resistant to HLB and Canker: Ongoing Project already funded by FCPRAC	$104,325

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Model systems, including chemical screening and therapeutics development				
Chung, K-R.		NIFA (CSREES)	Pharmaceutical approaches to prevent callose deposit and phloem blockage in citrus infected by the greening pathogen	
Gottwald, T.	USDA-ARS USHRL	FCPRAC	A mathematical modeling approach for the assessment of HLB disease-vector dynamics and management	N/A
Mou, Z.	UF-IFAS	FCPRAC	Transferring Disease Resistance Technology from a Model System to Citrus	$100,000
Powell, C.	UF-IFAS	FCPRAC	A Rapid Screening Process for Chemical Control of Huanglongbing	$75,730
Roose, M.	UC Riverside	Internal	Chemical Genomics Approach to HLB	N/A
Zhang, M.	UF/IFAS Indian River Research and Education Center	FCPRAC	Establishment of Novel System for evaluating effect of chemical compounds on citrus HLB	N/A
Unclassified				
Alvarado, A. N.	University of Puerto Rico Extension	NIFA (CSREES)	Puerto Rico Citrus Greening Training Program	
Browning,			UF IFAS Citrus Greening and Canker Research and Education Phase Four	

Principal Investigator	Affiliation	Funding source	Project Title/Research Topic	Budget Year One
Citrus Mutual			HLB Conference	
Grafton-Cardwell, E.	UC Cooperative Extension	NIFA (CSREES)	E-IPM Support Project, CA: Education Program for California and Arizona Stakeholders Affected by Asian Citrus Psyllid and Huanglongbing	

[a]Acronyms:

ACIAR	Australian Centre for International Agricultural Research
CACSM	Central American Cornon Market
CECNEA	Chamber of Exporters of Citrus in northeastern Argentina
CHRP	Citrus Health Response Program
CITF	Citrus Industry Trust Fund
CNPq	National Council of Scientific and Technological Development, Brazil
CPHST	Center for Plant Health Science and Technology
CRB	Citrus Research Board
CREC	Citrus Research and Education Center
CSREES	Cooperative State Research, Education, and Extension Service (USDA)
CWB	California Walnut Board
DOC	Department of Citrus
EMRAPA	Brazilian Agricultural Research Corporation
FAPESP	Foundation for Research Support of São Paulo
FDWSRU	USDA Foreign Disease-Weed Science Research Unit
FFVA	Florida Fruit and Vegetable Association
GR	general revenue
HLB	huanglongbing
IFAS	Institute of Food and Agricultural Sciences (USDA)
INTA-EEA	Instituto Nacional de Technologia Agropecuaria-Estacion Experimental Agropecuaria
IRCL	Indian River Citrus League
NAA	North Atlantic Area (USDA ARS)
NIFA	National Institute of Food and Agriculture (USDA)
NRI	National Research Initiative (USDA
NSF	National Science Foundation

NVDMC	New Varieties Development and Management Corporation
PPQ	Plant Pest and Quarantine
SBIR	Small Business Innovation Research
UC	University of California
USDA	US Department of Agriculture
USHRL	US Horticultural Research Laboratory (USDA)

Appendix K

Huanglongbing Research Milestones (1956–2009)

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
1956	Infectious nature of huanglongbing disease (HLB) was demonstrated.	Lin Kung Hsiang	Acta Phytopathologica Sinica 2:1-42
1957	This is the only known report on Lin Kung Hsiang's work to have appeared in the western world soon after the publication of Lin's work in 1956 (See: Lin, K.H., 1956). Unfortunately, Ciccarone's report remained essentially unknown too.	Ciccarone	Revista di Agrumicoltura 2: 45-50.
1965	Graft and insect vector (*Trioza erytreae*) transmissibility of greening was demonstrated.	McClean and Oberholzer	South Africa Journal of Agricultural Science 8:253-276; 297-298
1967	Transmission of HLB by the Asian citrus psyllid (ACP) was demonstrated.	Martinez and Wallace	Plant Disease Reporter 51:692-695

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
1967	With the use of psyllid to transmit the HLB pathogen, it was confirmed that trees with "citrus dieback" symptoms were positive for HLB.	Capoor et al	Indian Journal of Agricultural Sciences 37(6):572-576
1968	Citrus dieback in India is reported to have many similarities with greening disease of South Africa. The similarities with huanglongbing in China could not have been mentioned because Lin's work on HLB was still not known out of China.	Fraser and Singh	Proceedings of 4th Conference, IOCV:141-144, University of California, Division of Agricultural Sciences, Riverside, CA.
1968	Localized pockets of necrotic phloem were found scattered throughout vascular system of mature leaves in greening-affected sweet orange shoots in South Africa. Leaf mottle associated with HLB are thought to be caused by the reaction to the blockage of the translocation stream.	Schneider	Phytopathology 58:1155-1160
1969	HLB in Africa was found to be heat-sensitive and occurs only in areas below 30-32°C.*Trioza erytreae,* the African psyllid vector, was also found to thrive only in cool environments.	Catling	Journal of Entomology Society South Africa 32:209-223;
1970	"Mycoplasma-like organism" observed in citrus phloem tissue infected with HLB through electron microscopy.	Laflèche and Bové	Comptes Rendus de L'Academie des Sciences, Paris, 270:1915-1917
1970	These are the first two reports on bacterial structures associated with HLB, a disease until then considered to be caused by a virus. The structures were restricted to the phloem sieve tubes and were thought to be mycoplasmas, i.e bacteria lacking a cell wall. They were observed not only with South Africa greening, but also with Reunion and Indian greening. They were shown only a few months later to be not mycoplasma-like (see Saglio et. al., 1971).	Laflèche and Bové	Fruits 25: 455-465 C.R. Acad. Sci. Paris 270:1915-17
1971	HLB bacterium seen in citrus with "likubin" disease.	Chen et al	Phytopathology 61:598

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
1971	Citrus stubborn disease is also associated with sieve tube-restricted bacterial structures. These structures could be cultured and shown to be mycoplasma-like, surrounded by a 70 A° thick cell envelope, which lacked a cell wall and was composed only of a unit membrane. Compared to the stubborn mycoplasma-like structures, those associated with greening had a 200A° thick envelope and therefore could not be mycoplasma-like. The 200A° envelope was characteristic not only of South African greening, but also of the disease in Reunion, the Philippines, and India.	Saglio et al.	Physiologie Végétale 9: 569-82.
1971	As greening was found to be associated with bacteria, antibiotics, such as tetracyclines, were injected into affected trees in an effort to control the disease.	Schwarz and van Vuuren	Plant Disease Reporter 55: 747-750.
1973	HLB bacterium seen in citrus with "likubin" disease.	Tanaka and Doi	International Citrus Congress, Murcia-Valencia, pp. 352-353
1973	HLB bacterium seen in *T. erytreae.*	Moll and Martin	Phytophylactica 5:41-44
1973	HLB bacterium seen in *D. citri* (seen in salivary glands).	Chen et al.	Phytopathology 63(1):194-195
1973	HLB bacterium seen in citrus with "mottle leaf" disease.	Tanaka and Doi	International Citrus Congress, Murcia-Valencia, pp. 352-353
1974	First demonstration under phytotron conditions that African HLB was heat-sensitive (no symptom development at 32°C), while Asian HLB (from India, the Philippines) was heat tolerant (good symptom development at 32°C).	Bové et al.	Proceedings of 6th Conference, IOCV:12-15, University of California, Division of Agricultural Sciences, Riverside, CA.

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
1974	The cell envelope of the organism associated with greening was found to resemble that of a Gram-negative bacterium. However, the presence of peptidoglycan, a characteristic component of the bacterial cell wall, could not be demonstrated.	Moll and Martin	Proceedings of Conference Les Mycoplasmes/Mycoplasmas, INSERM 33: 89-96
1976	The African citrus psyllid vector, *Trioza erytreae*, is not only vector of the African HLB bacterium, but can also transmit the Asian HLB bacterium.	Massonié, Garnier, and Bové	Proceedings of 7th Conference (1976) IOCV: 18-20, University of California, Division of Agricultural Sciences, Riverside, CA.
1977	Through electron microscopy, the HLB causal organism was shown to possess a cytoplasmic membrane and a bacterial cell wall.	Garnier and Bové	Fruits 32:749-752
1980	Greenhouse-grown HLB-affected citrus plants having absorbed penicillin through the roots grew better, produced more roots and larger symptomless shoots and leaves than untreated controls. In contrast, Penicillin had no effect on Stubborn-affected citrus plants. A beneficial effect was also noted when penicillin was injected into the trunk of field-grown HLB-affected sweet orange trees in Reunion Island. Tetracycline had a beneficial effect on HLB-affected citrus as well as on stubborn-affected citrus. In view of the mode of action of penicillin (inhibits late step in peptidoglycan biosynthesis) and tetracycline (inhibits protein biosynthesis), these results strongly suggested that the bacterium associated with greening contained peptidoglycan, the characteristic component of the bacterial cell wall, indicating that the greening organism is a walled bacterium.	Aubert and Bové; Bové et al.	Proceedings of 8th Conference, (1980) IOCV: 103-108; 91-102 University of California, Division of Agricultural Sciences, Riverside, CA.

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1980	First successful biological control of *Trioza erytreae* and *Diaphorina citri*, the two psyllid vectors of HLB, in Reunion Island.	Etienne and Aubert	Proceedings of 8th Conference, (1980) IOCV: 118-121 University of California, Division of Agricultural Sciences, Riverside, CA.
1983	First transmission of HLB from citrus to periwinkle through dodder. In periwinkle, like in citrus (see: Bové et al., 1974), the Asian HLB bacterium was found to be heat tolerant, while the African HLB bacterium was heat sensitive, showing that the temperature effect was due to the bacterium and not to the host. These observations were the first indications that the two bacteria were biologically different.	Garnier and Bové	Phytopathology 73: 1358-1363
1984	The HLB-associated bacterium was shown to possess a cell wall of the Gram-negative type with an outer cell wall membrane and a peptidoglycan layer, thus establishing the Gram negative nature of the HLB-associated bacterium.	Garnier, Danel and Bové	Annales de l'Institut Pasteur. Microbiologie 135(1): 169-179; Proceedings of 9th Conference, IOCV: 115-124 University of California, Division of Agricultural Sciences, Riverside, CA.
1986	The ACP vector, *Diaphorina citri*, is not only vector of the Asian HLB bacterium, but can also transmit the African HLB bacterium. Since *Trioza erytreae* can also transmit the two bacteria (see: Massonié et al., 1976), each psyllid can transmit each one of the two bacteria.	Lallemand, Fos, and Bové	Fruits 41: 341-343
1991	First extensive review on citrus greening disease was published.	Da Graça	Annual Review of Phytopathology 29:109-36

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
1992	By random cloning of total DNA from periwinkle plants affected by Indian HLB, the first genome fragments of the HLB-associated bacterium were obtained: In-2.6, In-1.0, and In-0.6. Sequencing showed In-2.6 to be the nusG-rplKAJL-rpoBC gene cluster (beta operon), and In-1.0 coded for a bacteriophage DNA polymerase. In-2.6, used as a hybridization probe, detected all Asian HLB strains but not the South African strain.	Villechanoux et al.	Current Microbiology 24: 89-95; Current Microbiology 26: 161-166.
1993	Monoclonal antibodies specific for HLB bacteria were produced.	Gao et al.	Proceedings of 12th Conference, (1993) IOCV: 244-249, University of California, Division of Agricultural Sciences, Riverside, CA
1994	On the basis of 16SrDNA sequence analyses, the HLB-associated bacterium was confirmed to be a Gram negative bacterium, belonging to a new genus, *Candidatus* Liberobacter, in the alpha subdivision of the Proteobacteria. "*Candidatus*" indicated that the HLB-bacterium was not available in culture. Two liberobacter species were characterized by molecular, biological, and serological methods: *Candidatus* Liberobacter africanum (*C*Laf) in Africa and *Candidatus* Liberobacter asiaticum (*C*Las) in Asia.	Jagoueix, Bove, and Garnier	International Journal of Systematic Bacteriology 44: 397-86
1993	Transmission of HLB to tobacco by dodder was demonstrated.	Garnier and Bové	Proceedings of 8th Conference, (1993) IOCV: 212-219, University of California, Division of Agricultural Sciences, Riverside, CA
1995	Part of the rplKAJL-rpoBC operon of the African liberobacter was obtained as a 1.7 kb fragment (As-1.7). As-1.7, used as a probe, detected the African HLB strains, but not the Asian strains.	Planet et al.	Current Microbiology 30: 137-141

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
1996	First PCR detection of the Asian and African liberibacters by amplification of 16S rDNA.	Jagoueix, Bove, and Garnier	Molecular and Cellular Probes 10: 43-50.
1996	Already known to harbor both the African and the Asian psyllid vectors of HLB, Reunion and Mauritius islands were also shown to carry both the African and the Asian liberibacters.	Garnier et al.	Proceedings of 13th Conference, IOCV: 392-394, University of California, Division of Agricultural Sciences, Riverside, CA
1997	Confirmation that *C*Las and *C*Laf are two different bacterial species based on the 16S/23S rRNA intergenic regions.	Jagoueix et al.	International Journal of Systematic Bacteriology 47(1):224-227
1997	Petroleum spray oil was tested against the ACP in Guangzhou, China; It was found that petroleum oil was as effective as an organophosphate pesticide and an insect growth regulator in controlling psylla nymphs.	Rae et al.	International Journal of Pest Management 43(1):71-75
1999	Random Amplified Polymorphic DNA (RAPD) was used to identify additional genomic sequences of the HLB liberibacters. In particular, the omp (outer membrane protein) gene was obtained.	Hocquellet, Bove, and Garnier	Hocquellet, A., Bové, J.M., and Garnier, M. 1999. Isolation of DNA from the uncultured "*Candidatus* Liberobacter" species associated with citrus huanglongbing by RAPD.
1999	A PCR detection method based on the amplification of ribosomal protein genes, which allows direct identification of the liberibacter species by the size of the amplified DNA, was developed.	Hocquellet et al.	Molecular and Cellular Probes 13(5):373-379

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2000	The Asian psyllid vector of HLB, *Diaphorina citri*, was captured for the first time in southern Iran in 1997 during a mission on Witches' Broom Disease of Lime.	Bové et al.	Proceedings of 14th Conference, IOCV: 207-212 University of California, Division of Agricultural Sciences, Riverside, CA
2000	Following a 1999 proposal in prokaryote nomenclature, “Liberobacter”, “africanum”, and “asiaticum were respectively renamed “Liberibacter”, “africanus”, and “asiaticus”. A new liberibacter was detected in an ornamental rutaceous tree, *Calodendron capense*, in South Africa. The liberibacter was identified as a subspecies of Ca. L. africanus and named “*Ca.* L. africanus subsp. Capensis”.	Garnier et al.	International Journal of Systematic and Evolutionary Microbiology 50: 2119-2125.
2000	The HLB causal agent was shown to replicate in Chinese box orange (*Severinia buxifolia*) and wood apple (*Limonia acidissima*) but not in common jasmine orange (*Murraya paniculata* var. *paniculata*) and curry leaf (*Murraya euchrestifolia*).	Hung et al.	Journal of Phytopathology-Phytopathologische Zeitschrift 148(6):321-326
2000	This study was conducted to determine the effect of temperature on the biology and life table parameters of the ACP. Findings include the following: 1) Average number of eggs produced per female significantly increased with increasing temperature; maximum number of eggs was produced at 28°C; 2) Population reared at 28°C had the highest intrinsic rate of increased and net reproductive rate and the shortest population doubling time and mean generation time compared with populations reared at 15-25°C; 3) The optimum range of temperature for *D. citri population* growth was found to be 25-28°C.	Liu and Tsai	Annals of Applied Biology 137(3):201-206

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2000	The endosymbiotic microbiota of the citrus psyllid was investigated using PCR and RFLP. The whole DNA of *D. citri* was found to contain sequences that are similar to that of mycetocyte symbionts of other psyllids, Oxalobacter and Herbaspirillum, Arsenophonous spp., Liberobacter spp. and Wolbachia spp.	Subandiyah et al.	Zoological Science 17:983-989
2000	Taylor's power law and Iwao's patchiness regression models indicated that *D. citri* populations were aggregated.	Tsai et al.	Florida Entomologist 83(4):446-459
2001	The ability of *D. citri* and its two parasitoids, *Tamarixia radiata* and *Diaphorencyrtus aligarhensis* to survive at different relative humidities and temperatures was studied. This study showed that *D. citri* survived longer than the parasitoids at all conditions tested, indicating a lower net water loss rate.	McFarland and Hoy	Florida Entomologist 84(2): 227-233
2002	Psyllid population levels on orange jasmine were found to be positively related to the availability of new shoot flushes, which were in turn related to the weekly minimum temperature and rainfall. The study indicated that continuous flushes produced by orange jasmine could play an important role in maintaining high populations of psyllids when new shoot flushes are not available in citrus groves.	Tsai et al.	Florida Entomologist 85(3):446-451
2004	First report of *C*Las associated with HLB in Brazil.	Colleta-Filho et al.	Plant Disease 88:1382.
2004	Using PCR, it was determined that Liberibacter asiaticus persists in the Asian citrus psyllid vector but it is not transovarially transmitted.	Hung et al.	Plant Pathology 53(1):96-102

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2004	Results from a laboratory and spray booth study indicated that nymphal and adult *D. citri* and a mite complex can be controlled by high concentrations of sucrose octanoate, a synthetic analog of natural sugar esters found in leaf trichomes of wild tobacco (*Nicotiana gossei* Domin).	McKenzie and Puterka	Journal of Economic Entomology 97(3):970-975
2004	Coccinellid beetles were found to be the most important biological control agents of high-density populations in central Florida and that intraguild predation causes >95% mortality of immature stages of *Tamarixia radiata.*	Michaud	Biological Control 29(2):260-269
2004	A rearing method was developed for ACP and its parasitoids, *Tamarixia radiata* and *Diaphorencyrtus aligerhensis*.	Skelley and Hoy	Biological Control 29(1):14-23
2005	Omp (outer membrane protein gene)-based PCR-RFLP analysis was shown to be a simple method for detecting and differentiating *C*Las isolates. The phylogeny tree based on the omp gene sequences of the African and Asian liberibacters was very similar to the tree based on the 16S rDNA sequences.	Bastianel et al.	Applied Environmental Microbiology 71:6473-6478.
2005	*Murraya paniculata*, the preferred host of the Asian HLB psyllid vector, was also found to be a host of the two HLB bacteria present in São Paulo State, *C*Lam and *C*Las.	Lopes, Martins, and Frare	Summa Phytopathologica 31: 48-49.; Fitopatologia Brasileira 31: 303.
2005	Discovery and first report of a new liberibacter species associated with HLB in São Paulo State, Brazil: *Candidatus* Liberibacter americanus. The new liberibacter was also detected in *Diaphorina citri*, suggesting that the Asian psyllid vector in Brazil was transmitting not only *C*Las, but also *C*Lam.	Texeira et al.	Plant Disease 89: 107; International Journal of Systematic and Evolutionary Microbiology 55: 1857-1862; Proceedings of 16th Conference, IOCV: 325-340 University of California, Division of Agricultural Sciences, Riverside, CA

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2005	Development of PCR techniques for the detection of *C*Lam and *C*Las in citrus and psyllids.	Texeira et al.	Molecular and Cellular Probes, 19; 173-179; Proceedings of 16th Conference, IOCV: 432-438 University of California, Division of Agricultural Sciences, Riverside, CA
2005; 2007	*C*Las was detected in Wampee (*Clausena lansium* Skeels) using nested PCR.	Ding et al.; Deng et al.	Journal of Plant Pathology 87(3):207-212; Plant Health Progress 2007
2005	Detection of HLB pathogen through loop-mediated isothermal amplification (LAMP) was demonstrated; The LAMP method, which does not use a thermocycler and electrophoresis apparatus, is deemed useful for under-equipped laboratories.	Okuda et al.	Plant Disease 89(7):705-711
2005	Coccinellid species found to have potential important role as predators of the psyllid in Puerto Rico.	Pluke et al.	Florida Entomologist 88(2):123-128
2005	Third bacterial species was detected and identified as another causal organism of HLB in Brazil. The name *Ca.* Liberibacter americanus was proposed for this HLB pathogen. Detection of *C*Lam in *D. citri* indicated that this is also the vector for this liberibacter species.	Teixeira et al.	Molecular and Cellular Probes 19(3):173-179; International Journal of Systematic and Evolutionary Microbiology 551857-1862 Part 5
2005	In greenhouse trials, a neem-based biopesticide containing 4.5% azadirachtin was found to reduce psyllid nymph populations; however, no mortality was observed in psyllid adults that were exposed to 11-180 ppm azadirachtin.	Weathersbee and McKenzie	Florida Entomologist 88(4):401-407
2006	Guava intercropping was observed to reduce ACP/HLB incidence in Vietnam	Beattie et al.	Unpublished

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2006	The first extensive review on HLB following arrival of the disease in America was published.	Bové	Journal of Plant Pathology 88: 7-37.
2006	TaqMan real-time PCR using 16S rDNA-based TaqMan primer-probe sets specific to the different *Ca.* L. spp. was developed. Used successfully in the confirmation of HLB caused by *Ca.* L. asiaticus in FL.	Li et al.	Journal of Microbiological Methods 66(1):104-115
2006	Conventional PCR and two real-time PCR (RTi-PCR) methods were developed and compared according to their sensitivity and specificity for detecting *C*Las. The SYBR Green I (SGI) RTi-PCR was found to be most sensitive; the TaqMan RTi-PCR assay was rapid and had the greatest specificity. Mottled leaves yielded highest positive rate, indicating that leaf mottling was the most reliable symptom for field surveys.	Wang et al.	Plant Pathology 55(5):630-638
2006	First demonstration that *Diaphorina citri* transmits *C*Lam in São Paulo State, Brazil.	Yamamoto et al.	Page 96 in: Proceedings of the Huanglongbing Greening International Workshop, Ribeirão Preto, SP, Brazil.
2007	This study showed that maximum and minimum temperatures are more important than mean temperature in determining winter survival of *D. citri; D. citri* will be able to survive winter in areas with mean daily minimum and maximum temperatures of 5° and 12.5 °C.	Ashihara	Japanese Journal of Applied Entomology and Zoology 51(4): 281-287
2007	Polymorphic microsatellite markers were developed from micro-satellite-enriched DNA libraries and mined from an expressed sequence tags library of *D. citri*. Microsatellite loci can provide means for assessing overall genetic variation and migration patterns for *D. citri*.	Boykin et al.	Molecular Ecology Notes 71202-1204

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2007	It was found that yellow sticky card traps and blue sticky card traps were equally effective in detecting the presence of *D. citri* adults in trees while CC traps (red, blue, black, white, yellow, and dark green bases) and multi-lure traps captured few adults and provided poor detection of adult psyllids. Tap sampling was found to be an easy way to conduct and provided relatively good detection of trees infested by adults, based on the level of infestation in the two groves that were sampled in this study. Stem tap sampling provides information on the presence and relative abundance of adult *D. citri* in one visit to a block of trees while sticky trap sampling requires two visits.	Hall et al.	Florida Entomologist 90(2):327-334
2007	A sampling procedure was devised to estimate flush abundance. It was found, based on Taylor's power law coefficients, that over all sample weeks, flush shoots were randomly distributed within the young grapefruit trees and only weakly aggregated within the block of mature orange trees. Projections indicated that a sampling plan of 40 trees (one sample per tree) would provide density estimates that are acceptable enough for general estimates at mean densities of one or more shoots per sample.	Hall and Albrigo	HortScience 42(2):364-368
2007	It was found that 1) a 3% (wt:vol) suspension of kaolin-based hydrophilic particle film (Surround WP) in water was not acutely toxic to eggs, older nymphs or adults, 2) presence of dried particle on leaves hampered the ability of adults to grasp and walk on citrus leaves, 3) numbers of eggs and nymphs per flush shoot were reduced by 85% and 78%, respectively, in trees treated with particle film. However, the suppressive effects of Surround treatment were degraded by rain.	Hall et al.	Journal of Economic Entomology 100(3):847-854

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2007	TaqMan real-time PCR was found to be 10 to 100-fold more sensitive than conventional PCR and LAMP; It can be used as a tool for early detection of HLB and identification of *C*Las before symptom development.	Li et al.	Plant Disease 91(1):51-58
2007	Removing HLB-affected branches from symptomatic trees ("pruning"), in an effort to decrease the sources of HLB inoculum, has been found ineffective in São Paulo State to control HLB and should not replace removal of the whole trees.	Lopes et al.	European Journal of Plant Pathology 119: 463-468.
2007	This study determined the duration and viability of the egg and nymphal stages, sex ratio, fecundity, and longevity of *D. citri* on three hosts (orange jasmine, Rangpur lime, and Sunki mandarin). *D. citri* lower temperature threshold and thermal constant values for the egg, nymphal, and biological cycle were determined.	Nava et al.	Journal of Applied Entomology 13(1):709-715
2007	The reproductive biology and behavior of *D. citri* was studied; Emergence patterns of males and females were found to be similar, with no evidence for protandry or protogyny. Both males and females reached reproductive maturity by 2-3 days post eclosion. Oviposition generally began within 1 day after mating, but was longer when females mated at 2d of age. Mating on orange jasmine was observed almost exclusively on flush shoots during daylight hours, with no obvious peak of daily mating activity. Mating activity may be constrained during scotophase partly due to cooler temperatures and lack of light.	Wenninger and Hall	Florida Entomologist 90(4):715-722
2007	First report of dodder transmission of HLB from naturally infected *Murraya paniculata* to citrus.	Zhou et al.	FCPRAC; Plant Disease 91(2):227-227

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2008	First study of transcriptional profiling in citrus in response to liberibacter infection using microarray technology. Results indicate that gene expression changes involved a variety of different processes including cell defense, transport, cellular organization, photosynthesis, and carbon metabolism. Pathogen-induced accumulation of transcripts for phloem-specific lectin PP2-like protein was noted.	Albretch and Bowman	Plant Science 175(3):291-306
2008	Factors involved in HLB management in 20 citrus farms in São Paulo State over a period of four years (2004 to 2008) have been identified and explain success or failure of HLB control.	Belasque et al.	International Research Conference, Huanglongbing, Dec. 1-5 , Orlando, FL
2008	It was determined that the HLB bacterium may be translocated into the external parts of the embryo during seed development but this infection typically disappears during early growth and thus unlikely to result in seedlings that have HLB disease symptoms or that can serve as sources of inoculum.	Bowman	Manuscript submitted for publication; FCPRAC Annual Report 2008
2008	Transformation of citrus with AMP (antimicrobial peptide) D4E1 (synthetic derivative of cecropin) and the garlic leaf lectin, ASAL, was carried out. Transformation is complete and transformants with the AMP are now being grown in the greenhouse for testing against HLB and canker.	Bowman	FCPRAC Annual Report 2008
2008	Laboratory trials showed that Silwet L-77 and Kinetic (organosilicone adjuvants) alone and petroleum oil and copper hydroxide alone, or in combination with the adjuvants, had low residual and acute toxicity to *T. radiata* (parasitoid of *D. citri*) and appear to be compatible with biocontrol using *T. radiata*.	Cocco and Hoy	Florida Entomologist 91(4):610-620
2008	On the basis of PCR data, co-cultivation of *C*Las with Actinobacteria was reported. No pure cultures of liberibacters could be obtained.	Davis et al.	Plant Disease 92: 1547-1550

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2008	A transient-expression vector based on Citrus tristeza virus (CTV) that provides systemic expression of foreign genes in citrus was built. The CTV-based vector is being developed as an interim measure to protect citrus trees. It has continually expressed foreign genes in citrus for six years so far. 27 of 30 citrus trees that have been infected with the vector for 4-6 years still contain the intact foreign gene. Based on this observation, it is believed that a very high percentage of the trees would retain the peptide for ten years or more.	Dawson	FCPRAC Annual Report 2008
2008	It was determined that the non-tRNA gene regions of the 16S-23S ITS and the 23S-5S rRNA ITS of *C*Las did not share any similarity to any known non-Liberibacter DNA sequences and could be used for the specific and efficient detection of *C*Las.	Deng et al.	Molecular and Cellular Probes 22(5-6):338-340
2008	Differences in the microbial community composition of HLB-infected and recovered plants and Las-infected and non-infected psyllids were detected with the use of 16S rDNA-based molecular techniques and traditional culturing methods. Species of bacteria that support the multiplication of Las bacterium on agar media plates were tentatively identified as *Aerations altamirensis*, *Phycibacter jejuensis* and *Agrococcus versicolor* based on their 16S r DNA analyses. RFLP and library-based assays of the partial RNA operon (~3.3kb) of Las bacteria indicated the genetic diversity in the Las bacteria that are present in plants that displayed different disease phenotypes. Subsequent sequence analysis indicated that all these variations belonged to single nucleotide polymorphisms (SNPs) and the SNPs varied from population to population.	Duan	FCPRAC Annual Report 2008
2008	Transmission of HLB from citrus to tomato plants via dodder was confirmed.	Gabriel and Duan	FCPRAC Annual Report 2008

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2008	More than 265 transgenic plants from independent transformation events with six different anti-bacterial constructs were regenerated, including many sweet orange and grapefruit, several with a phloem-limited promoter. All are being micro-grafted for subsequent HLB challenge. The first transgenic grapefruit plants containing the LIMA construct, challenged (greenhouse) with HLB over a year ago, remain RT-PCR negative with no symptoms, while the grafted HLB inoculum budsticks remain RT-PCR positive; this encouraging result suggests that LIMA may provide resistance or at least substantially delay disease.	Gmitter	FCPRAC Annual Report 2008
2008	Development of promoters that will prevent expression of foreign genes in fruit; demonstrated the feasibility of engineering zinc finger-based proteins to regulate the expression (up to 77% inhibition) of genes used currently to produce antibacterial proteins.	Gurley	FCPRAC Annual Report 2008
2008	It was found that in Florida, *D. citri* was most consistently present and more abundant at the study sites in the months of May, June, and July. Results of this study also indicate that large infestations could occur anytime of the year depending on environmental conditions and flush availability.	Hall et al.	Environmental Entomology 37(4):914-924
2008	The latency period of Liberibacter in adult *D. citri* was determined to be 8-16 weeks	Hall	FCPRAC Annual Report 2008

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2008	Experiments with *Diaphorencyrtus aligarhensis* (parasitoid of ACP) imported from China showed that this strain is ideally suited for mass rearing and release into Florida as a biological control agent. Choice and no choice tests indicated that this parasitoid will parasitize 2nd thru 4th instar nymphs only and will host feed on 1st thru 4th instars of immature psyllids. Y-tube experiments show the parasitoids are not attracted to various uninfested host plants but are attracted to ACP-infested plants, psyllid nymphs and honeydew/wax waste products from nymphal feeding.	Hall	FCPRAC Annual Report 2008
2008	First description of a non-citrus liberibacter transmitted by the tomato/potato psyllid, *Bactericera cockerelli*. The new liberibacter has no association with HLB.	Hansen et al.	Applied and Environmental Microbiology 74: 5862-5865.
2008	Two PCR primer sets (16S and a pair of 10F, 489R) were chosen to detect novel endosymbiont bacterial 16s rDNA sequences within the ACP. Sequence homology results found 40 of the 47 were 100% homologous to a Syncytium endosymbiont of *D. citri* (accession number EF433792) and seven were 100% homologous to Wolbachia of *D. citri* (accession number EF433793). The 2.5 kb of PCR product with 10F, 489R primer pairs contained six major types of sequences. These methods identified the presence of eight different bacterial homologs: Carbazole degrading bacterium, Janthinobacterium sp. IC161, an ammonia-oxidizing bacterium, Nitrosospira multiformis ATCC25196, known as a biodegradation bacterium, Acinetobacter sp. and an alkane degrading bacterium, Alkanindiges. These data provide evidence that Asian citrus psyllids are supported by a rich bacterial fauna of many endosymbiotic and gut fauna bacteria of various types, all of which have important interactions between each other and may interact with *C*Las when it occurs in psyllids.	Hunter	FCPRAC Annual Report 2008

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2008	This study that evaluated nine different commercially available media for their suitability to culture psyllid cells. The most efficient medium determined so far is the SF900-III-supplemented. It was found that a combination of this media plus 10% Fetal bovine serum can support psyllid cells for over three months.	Hunter	FCPRAC Annual Report 2008
2008	Primary cell lines successfully established using *D. citri* embryos.	Keyhani	FCPRAC Annual Report 2008
2008	This study showed that standard curve established with DNA extracted from naturally-infected field-grown plants was more accurate than the standard curve constructed from plasmids containing the amplification targets as cloned inserts. A universal standard curve was established for the quantification of the pathogen in various citrus tissues of different citrus species planted in different geographic locations.	Li et al.	Plant Disease 92(6):854-861
2008	330 symptomatic and symptomless leaf samples were collected from HLB orchards in Guangdong, Guangxi, Fujian, Jiangxi, Zhejiang, Hunan, Yuannan and Guizhou provinces of China. While *Ca.* L. asiaticus was detected in 96 samples, *Ca.* L. americanus was found in only one sample, a sample from Hunan. If this result is confirmed, it will be the first time that *Ca*. L. americanus has been detected out of Brazil. Also, so far the American liberibacter as well as the Asian liberibacter have never been detected in Africa.	Lou et al.	Program and Abstracts book, 11th International Citrus Congress, p. 232, abstract P333.
2008	Morphological and genetic data was found to support the identification of the fungus that killed adult *D. citri* in a FL citrus grove in 2005 as *Isaria fumosorosea* (=*Paecilomyces fumoroseus*). Completion of Koch's Postulates established the pathogenicity of the fungus to *D. citri*.	Meyer et al.	Journal of Invertebrate Pathology 99(1):96-102

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2008	Micronized fluorescent powder found to be effective for marking ACP; does not affect survival and can be used for ca. 5 weeks in the field.	Nakata	Applied Entomology and Zoology 43 (1):33-36
2008	The external morphology of the antennal sensilla of male and female psyllids were examined using scanning electron microscopy and the putative functions of the identified sensilla were determined using transmission electron microscopy techniques.	Onagbola et al.	Micron 39(8):1184-1191
2008	Aldicarb application (5.6 kg/ha) to the bed side of mature citrus trees 2-3 months before spring growth can suppress ACP through spring without direct effects on principal psyllid natural enemies. This practice is now widely adopted by the industry.	Qureshi and Stansly	Pest Management Science 64(11):1159-1169; FCPRAC Annual Report 2008
2008	A volatile (dimethyl disulfide or DMDS) was discovered for the first time from guava leaves. DMDS is known to have potent repellency and insecticidal properties against other insects.	Rouseff et al.	FCPRAC Annual Report 2008; Journal of Agricultural and Food Chemistry 56(19):8905-8910
2008	A study performed in 34 grapefruit and 6 sweet orange orchards from March to August in Southern Texas showed that densities of *D. citri* eggs, nymphs and adults were significantly higher in sweet orange than in grapefruit and that the number of *D. citri* immatures were significantly higher in the southern quadrant of the trees. The spatial distribution pattern was analyzed using Iowa's patchiness regression and Taylor's power law models. According to projections, a sampling plan consisting of 10 trees and 8 flush shoots per tree would provide acceptable density estimates of the three developmental stages of *D. citri* that can be used for population studies and for making management decisions.	Setamou et al.	Journal of Economic Entomology 101(4):1478-1487

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2008	Lab and field trials demonstrated that an organosilicone adjuvant (Silwet L-77 or Kinetic) added to one-fourth or one-half the lowest label rate for imidacloprid killed as many eggs, nymphs as the lowest label rate of imidacloprid but did not control adults as well as the lowest label rate.	Srinivasan et al.	Florida Entomologist 91 (1) 87-100
2008	This study demonstrated that aerial applications of broad-spectrum insecticides such as Imidan® (Phosmet) provided satisfactory control but that more selective insecticides such as Provado® (imidacloprid) and Delegate® (spinetoram) require better coverage obtained from ground applications. Resulting recommendations have been adopted by citrus and agrochemical industries.	Stansly and Qureshi	FCPRAC Annual Report 2008
2008	This study provided 1) direct proof that unmanaged citrus groves serve as reservoirs of psyllids that infest nearby commercial groves; and 2) direct proof of rapid short range psyllid movement which suggests that lack of control in a nearby grove can lead to rapid reinfestation in intensely-managed groves.	Stelinski	Unpublished; FCPRAC Annual Report 2008
2008	*C*Las was found to be distributed in bark tissue, leaf midrib, roots, and different floral and fruit parts, but not in the endosperm and embryo of infected fruits. HLB pathogen was found to be unevenly distributed in planta, with a relatively high concentration in fruit peduncles.	Tatineni et al.	Phytopathology 98(5):592-599
2008	The tufB-secE-nusG-rplKAJL-rpoB gene cluster of *C*Las could be obtained and sequenced, and compared to the gene cluster of *C*Laf and *C*Las. These comparisons confirmed that *C*Lam represented a distinct liberibacter species. Liberibacter speciation dating was also evaluated. The time of divergence between *C*Laf and *C*Las was estimated at 147 million years (Myr) and the splitting between *C*Lam and the asiaticus/africanus branch would have occurred 309 Myr ago.	Teixeira et al.	International Journal of Systematic and Evolutionary Microbiology. 58: 1414-1421.

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2008	Occurrence of *C*Lam was examined in 822 leaf samples from a single HLB- affected sweet orange tree by two conventional PCR techniques and a newly developed real time (RTi) PCR, also used for quantification of the liberibacter in the leaves. Even though RTi-PCR detected as few as 10 liberibacters per gram of leaf tissue (l/g), no liberibacters could be detected in any of the many leaf samples from a symptomless branch, while in blotchy mottle leaves from symptomatic branches of the same tree, the liberibacter titer reached values as high as 107 l/g. Nested PCR was almost as sensitive as RTi-PCR.	Teixeira et al.	Molecular and Cellular Probes 22: 139-150.
2008	PCR and RTi-PCR were used to detect/quantify *C*Lam in citrus; the study showed that the pathogen was unevenly distributed in HLB infected citrus trees.	Teixeira et al.	Molecular and Cellular Probes 22(3):139-150
2008	A phytoplasma of group IX is associated with citrus HLB symptoms in São Paulo State, Brazil.	Teixeira et al.	Phytopathology 98(9):977-984
2008	Sweet orange trees showing characteristic leaf and fruit symptoms of HLB in São Paulo State, but testing negative for all known liberibacters, were found to be infected with a phytoplasma of group 16Sr IX, closely related to the Pigeon pea witches' broom phytoplasma. The phytoplasma could be detected easily in citrus by PCR using universal or specific primers. The HLB-associated phytoplasma could also be detected in *Crotalaria junceae* plants (grown in between rows of citrus trees for improvement of soil conditions) and showing typical witches' (Wulff et al., unpublished). The insect vector transmitting the phytoplasma to citrus trees, probably from the infected Crotalaria plants, has not yet been identified.	Teixeira et al.	Phytopathology 98: 977-984.

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2008	Isothermal and chimeric primer-initiated amplification of nucleic acids combined with cycling probe technology (cycleave ICAN) was found to shorten the time for large-scale detection of HLB.	Urasaki et al.	Journal of General Plant Pathology 74(2):151-155
2008	Behavioral evidence was found for a female-produced volatile sex attractant pheromone in *D. citri.*	Wenninger et al.	FCPRAC; Entomologia Experimentalis et Applicata 128 (3):450-459
2008	It was demonstrated that *D. citri* females require multiple matings over time to achieve high reproductive output, but oviposition is constrained by the presence of males.	Wenninger and Hall	Physiological Entomology 33(4):316-321
2009	Laboratory trials showed the effect of temperature on the toxicity of selected organophosphates, carbamate, avermectin, pyrethroid, and neonicotinoid insecticides against adult *D. citri*. Data collected can be used when selecting insecticides depending on the prevailing environmental temperature.	Boina et al.	Journal of Economic Entomology 102 (2):685-691
2009	Updated review on HLB at the web site of IOCV (International Organization of Citrus Virologists) with 152 color illustrations on most aspects of HLB.	Bové	Bové, J.M. 2009. Citrus Diseases, Huanglongbing, Text and Image Gallery. http://www.ivia.es/iocv
2009	A phytoplasma related to *Ca.* Phytoplasma asteri was detected in citrus showing HLB symptoms in Guangdong, China.	Chen et al.	Phytopathology 99(3):236-242
2009	A complete circular genome has been obtained by metagenomics, using the DNA extracted from a single *C*Las-infected psyllid. The 1.23 Mb genome has an average 36.5% GC content. Many characteristics derived from the annotation are reported. The authors indicate that this is the first genome sequence of an uncultured α-proteobacterium that is both an intracellular plant pathogen and insect symbiont.	Duan et al.	Molecular Plant-Microbe Interactions 22: 1011-1020.

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2009	First report of HLB in the southern and southwestern parts of Iran. The Asian psyllid vector was detected in the area in 1997 (see Bové et al., 2000).	Faghihi et al.	Plant Pathology 58: 793.
2009	Microarray analysis indicated that HLB infection significantly affected the expression of 624 genes whose encoded proteins fell into the following categories: sugar metabolism, plant defense, phytohormone, cell wall metabolism and 14 other categories. Anatomical examination indicated that HLB bacterium infection caused phloem disruption, sucrose accumulation, and plugged sieve pores. It was determined that HLB-associated blockage resulted from plugged sieve pores and not due to HLB bacterial aggregates since *C*Las does not form aggregates in citrus.	Kim et al.	Phytopathology 99(1):50-57
2009	Systematic quantification of the distribution of *C*Las genomes in tissues of six citrus species using a quantitative polymerase chain reaction assay was performed. The study showed the ubiquitous presence of *C*Las in symptomatic citrus trees and the *variation in distribution* between individual trees and among samples of different tissues from the same trees.	Li et al.	Phytopathology 99(2):139-144

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2009	While the new liberibacter described by Hansen et al.; see Hansen et al., 2008) as *Candidatus* Liberibacter psyllaurus was characterized from the tomato/potato psyllid, *Bactericera cockerelli*, the liberibacter described by Liefting et al. (2009a, b) as *Candidatus* Liberibacter solanacearum was characterized from tomato and other solanaceous plants. The two liberibacters represent one and the same organism: *Ca.* L. psyllaurus refers to the agent in the psyllid vector, Ca. L. solanacearum refers to the agent in plants. It is the agent associated with potato Zebra chip disease (PZCD) and the PZCD psyllid vector. It has been shown that the citrus liberibacter, *C*Las, can experimentally infect, and induce disease in at least two solanaceous plants: tobacco (Garnier and Bové, 1993) and tomato (Duan et al., 2008). It might be expected that, similarly, *Ca.* L. solanacearum will be shown to infect, and induce symptoms in citrus.	Liefting et al.; Lin et al.	Plant Disease 93: 208-214; International Journal of Systematic and Evolutionary Microbiology 59: 2274-2276; Journal of Plant Pathology 91: 215-219.
2009	Percentages of transmission by graft inoculation were from 54.7 to 88.0% for *Ca.* L. asiaticus and 10.0 to 45.2% for *Ca.* L. americanus. Average bacterial titers in field trees were 6.67 log cells per gram of leaf midrib for *C*Las and 5.74 for *C*Lam. The titer of *C*Las in field trees being higher than that of *C*Lam, transmission of the former by the psyllid vector might be more efficient than that of the latter and explain why newly infected trees are more frequently infected with *C*Las than with *C*Lam.	Lopes et al.	Phytopathology 99: 301-306.
2009	While *C*Las, not yet affected at 35°C, was confirmed to be heat tolerant, *C*Lam, affected at 32°C, was found to be heat sensitive. Thus two of the citrus liberibacters are heat sensitive: *C*Laf and *C*Lam.	Lopes et al.	Plant Disease 93: 257-262.

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2009	Phylochip analysis indicated that 47 orders of bacteria in 15 phyla were present in citus leaf midribs while cloning and sequencing indicated 20 orders of bacteria in 8 phyla. Phylochip arrays indicated that nine taxa were significantly more abundant in symptomatic midribs than in asymptomatic midribs.	Sagaram et al.	Applied and Environmental Microbiology 75(6):1566-1574
2009	*C*Lam found to be less heat tolerant than *C*Las.	Lopes et al.	Plant Disease 93(3):257-262
2009	Cultivation of all three citrus liberibacter species has been reported. Confirmation in several laboratories is attempted.	Sechler et al.	Phytopathology 99:480-486.
2009	This study provides evidence that *D. citri* uses olfactory and visual cues in orientation to host plants which indicates the possibility of using plant volatiles to monitor and manage ACP.	Wenninger et al.	FCPRAC Annual Report 2008; Environmental Entomology 38(1):225-234
2009	Vibrational communication between male and female psyllids was studied. Findings include: 1) both sexes produced simple, low amplitude vibrational signals at multiples of 17-250 Hz, ranging in duration from 140 to 700 ms; 2) vibrational frequency in males were significantly negatively correlated with mass; 3) latent period for initialization of calling was significantly shorter for males exposed to clean air, suggesting that in the absence of olfactory cues, psyllids might be more inclined to use acoustic signals to communicate with conspecifics.	Wenninger et al.	Annals of the Entomological Society of America 102(3):547-555
2009	The size of the *C*Lam genome was determined by pulse field gel electrophoresis, using Lam-infected periwinkle plants for bacterial enrichment. The genome size was found to be ~1.31 Mbp, a value close to 1.23 Mbp as found by Duan et al., 2009. The data also suggest the genome to be circular and to contain three ribosomal operons.	Wulff et al.	International Journal of Systematic and Evolutionary Microbiology 59: 1984-1991.

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2009	Two field trials have been established in Florida to evaluate the resistance of transgenic citrus to citrus canker and citrus greening. The transgenic citrus tested carry the following genes: 1) gene encoding for a plant-based anti-microbial peptide and 2) a proprietary gene with the trademark "Disease Block". No field resistance data have been generated as of December 2009 but preliminary laboratory data for the transgenic citrus in both trials indicate efficacy against citrus greening.	M. Irey	http://www.aphis.usda.gov/biotechnology/status.shtml http://www.isb.vt.edu/cfdocs/fieldtests1.cfm
FCPRAC 2009 Progress Reports			
2009	Results from this study indicate that *D. citri* which complete their development on *C*Las(+) plants are more likely to acquire the pathogen compared to those individuals that feed on Las(+) plants as adults only. Therefore, the presence of *C*Las(+) trees on which psyllids can complete development is an important factor in the overall spread of HLB within a grove. Results from preliminary tests suggest that there appears to be some seasonality to the abundance of Las positive psyllids with one period of increase occurring in the late fall/early winter and a second smaller peak occurring in the spring.	Brlansky	FCPRAC Progress Report (January, 2009)
2009	Results from this study provided (further) evidence that distribution of the greening-associated pathogen varies widely within symptomatic, PCR-positive citrus trees and thus illustrates the importance of obtaining multiple samples from trees where an infection is suspected. Using real-time PCR, it was determined that 80% of stumps had one or more sprouts that were *C*Las positive. This work confirms the importance of controlling sprouts from citrus stumps in order to minimize the spread of HLB within and between citrus groves.	Brlansky and Davis	FCPRAC Progress Report (January, 2009); Proceeding of the International Research Conference on Huanglongbing. Orlando, FL

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2009	The response of 32 different citrus varieties or relatives to HLB was studied. The effect of HLB on the plants was differentiated most dramatically by the rate of continuing growth. Although some trees developed distinctive symptoms on leaves, growth was inhibited only marginally, while with other trees when leaves became chlorotic, growth ceased. Sweet orange, grapefruit, tangelo, and some mandarins were extremely sensitive. Preliminary results in terms of most severe symptoms and reduced growth are: Valencia > Hamlin > Rhode Red Valencia > Pineapple for sweet orange; for grapefruit Duncan = Marsh > Rio Red.	Dawson	FCPRAC Progress Report (January, 2009) See also Folimonova, S.Y., Robertson, C.J., Garnsey, S.M., Gowda, S., and Dawson, W.O. 2009. Examination of the responses of different genotypes of citrus to huanglongbing (citrus greening) under different conditions. Phytopathology 99(12):1346-1354
2009	DNA from highly infected citrus was sequenced. To date, we have 55 million bases from a 454 run with an average read length of 235 bases. We also have 255 million bases from a Solexa sequencer at Oregon State University. An additional 8 billion bases should be available within 2 weeks from a SOLiD sequencer at UF. All of these data will be assembled with expertise from the University of Florida and Oregon State University. Several assembly programs are being tested for this purpose. These data are expected to provide a significant amount of citrus genome data as well as the citrus greening genome. We are attempting to use these data to fill gaps in the sequencing results of other groups.	Dawson	FCPRAC Progress Report (January, 2009)
2009	Development and validation of a novel and efficient adaptor-based genomic subtractive enrichment protocol, which enables the nearly complete removal of host DNA and maximizes the likelihood of cloning only bacterial sequences from the extremely low titer of the bacterium present in infected tissues.	Gmitter	FCPRAC Progress Report (January, 2009)

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2009	The first transgenic grapefruit plants containing the LIMA construct were inoculated with HLB 18 months ago and remain RT-PCR negative with no symptoms, while the grafted HLB inoculum budsticks remain RT-PCR positive; this result suggests that LIMA may provide resistance or at least substantially delay disease. Citrus genes involved in systemic acquired resistance (SAR) have been identified and cloned, and several have already been engineered into transgenic plants, and are being tested against HLB and canker.	Gmitter	FCPRAC Progress Report (January, 2009)
2009	A sweet orange genome "re-sequencing project", using next-gen sequencing (454 of Roche Diagnostics) was initiated and is underway. This project is in concert with the ICGC sequencing initiative plans to use next-generation sequencing technologies on several diploid genomes, as an added resource to the Sanger haploid sequencing project conducted in parallel. Sequence reads already available have been aligned to 23 previously sequenced BAC clones, revealing excellent agreement between the previous Sanger sequence and the currently produced 454 sequence data.	Gmitter	FCPRAC Progress Report (January, 2009)
2009	It is estimated that for every tree that shows symptoms of HLB, there are on the average 13 trees (range is 2-52) that are infected but are asymptomatic. Latency of HLB is estimated to be from 6 to 36 months.	Gottwald	FCPRAC Progress Report (April, 2009)

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2009	A new strain of *Diaphorencyrtus aligarhensis* (parasitoid of ACP) was imported from China and is currently being reared and released to help reduce or eliminate ACP populations. Before release, temperature and relative humidity experiments, as well as parasitism rate experiments were conducted. Choice and no choice tests have shown that this parasitoid will parasitize 2nd thru 4th instar nymphs only and will host feed on 1st thru 4th instars of immature psyllids. Results from these experiments show that this strain is ideally suited for mass rearing and release into Florida as a biological control agent.	Hall	FCPRAC Progress Report (January, 2009)
2009	In this study, it was noted that fall and winter appear to be time periods when percentages of infected psyllids may be consistently the highest.	Hall	FCPRAC Progress Report (January, 2009)
2009	cDNA psyllid libraries were produced, which resulted in 17,000 expressed sequence tags. The libraries were from adult, testes, and midgut tissues. These results aided efforts to identify the microbial fauna of the Asian citrus psyllid, enabling genetic promoters to be designed for further analyses. Eight sequences suggest there is an as yet unidentified phage within the psyllids. Sequences from a new Reovirus were identified and characterized. Sequences from a new Wolbachia species were produced. The internal anatomy of the psyllid was identified for tissue collection in anticipation of future cDNA library constructions. An inbred psyllid population was created F7 to produce the needed genomic material for a psyllid genome effort. Several classes of cathepsins were identified, as well as a new FK506-binding protein; these proteins have important functions in body formation, digestion, and in egg/embryo metabolism, plus cell maintenance. Approximately 60 proteins and all other sequences have been published in the public database, at NCBI for use by the research community.	Hunter	FCPRAC Progress Report (January, 2009)

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2009	*D. citri* embryos were used to establish a series of primary cell lines and conditions for routine establishment of stable and transferable primary cell lines have been developed. To date, four cells lines Dce 10, Dce 17 and Dce 19(cell patch), and Dce 19(total) have undergone their first passage. All four cultures displayed cell growth after first passage of the cells into fresh media. Two cell lines, Dce 19 and Dce 17 have undergone their second passage, with clumps of cells appearing to grow. The current time of growth before passage is 4-5 weeks. We have successfully accomplished our second passage for two cells lines and anticipate continuing the cultivation of the cells. It is anticipated that efforts to begin cryopreservation of some cell aliquots will begin soon. Cell morphology of the various lines remains heterogeneous with some cells forming large clumps before spreading in the tissue culture flasks and whereas others remain in suspension.	Keyhani	FCPRAC Progress Report (January, 2009)
2009	Completion of assembly and annotation of all the Rutaceae sequences (ESTs and mRNAs) available in public databases (GenBank) and sequences from various previous studies (cold acclimation, canker response, salicylic acid treatment). With this information a new microarray based on Agilent technology was designed. Microarrays have been manufactured and a simple experiment (of known results) has been designed to test them before their release to the scientific community.	Moore	FCPRAC Progress Report (January, 2009)
2009	The baseline susceptibility information (LC50s) for commonly used insecticides for psyllid control was determined. These insecticides include the following: fenpropathrin (Danitol 2.4EC), chlorpyrifos (Lorsban 4E), carbaryl (Sevin XLR), imidacloprid (Provado 1.6F), dimethoate (Dimethoate 4EC), zeta-cypermethrin (Mustang), phosmet (Imidan), thiamethoxam (Actara; expected to be labeled for use in FL citrus in 2009) and abamectin (Agri-mek 0.15EC).	Rogers	FCPRAC Progress Report (January, 2009)

Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2009	Results from a laboratory study that tested the effect of spray droplet size on ACP control indicate that smaller droplet sizes result in greater mortality of all ACP life stages than larger ones suggesting that ULV sprays may give more effective control of ACP in the field. Tests conducted in the field showed that for insecticides that are known to be effective against ACP, low volume applications are as effective as standard airblast sprays for psyllid control. Dibrom, Micromite (nymph activity only), Malathion, Portal *(nymph activity only), Dimethoate, Provado 1.6 F, Mustang, Danitol, and Delegate are effective against ACP when applied as low volume sprays.	Salyani and Stelinski	FCPRAC Progress Report (January, 2009)
2009	In this study, it was noted that certain micro- and macro-element levels are significantly changed in greening infected trees. Data from this study also suggest that greening-induced nutritional changes may be detectable before the disease is detectable by PCR analysis	Spann	FCPRAC Progress Report (January, 2009)

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Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2009	Gamma-butyrolactone was identified as one of the behaviorally active compounds that likely comprise the pheromone of ACP. Testing in the laboratory has confirmed its behavioral activity to psyllids. This chemical is attractive to male psyllids and appears to repel females. Also, this chemical attracts the psyllid parasitoid, *Tamarixia radiata*. This information will be used to manipulate the parasitoids' behavior to enhance mass rearing programs of this species, a prerequisite to its utilization as a biocontrol agent. Biologically active compound(s), which attract *T. radiata*, will be incorporated into an efficient attractant formulation. Such attractants may allow for monitoring *T. radiata* population densities, which will allow for tailoring spray programs such that peak parasitoid population densities can be avoided and conserved. Also, deployment of a potent attractant within groves. Alpha Scents has formulated gamma-butyrolactone into lures as well as an attract-and-kill formulation for direct psyllid control, which we will be evaluating this year as psyllid populations build up.	Stelinski	FCPRAC Progress Report (January, 2009)
2009	CLas was found to be present in bark tissue, leaf midrib, roots and different floral and fruit parts but not in the endosperm and embryo of infected fruit. Quantification analysis showed that CLas is not evenly distributed in planta and a relatively high concentration was found in fruit peduncles. A method for quantifying viable CLas cells was developed. This method (EMA-QPCR), which uses ethidium monoazide to differentiate between live and dead cells, could provide an accurate assessment of the amount of viable CLas cells in plant samples	Wang	FCPRAC Progress Report (January, 2009); see also Satyanarayana et al. (Phytopathology, 2008)

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Year(s)	Milestone	Principal Investigator(s) or Author(s)	Publication/Journal
2009	Microscopy analysis was performed to compare the phloem of HLB infected citrus and healthy control. It was observed that 1) there was significantly greater accumulation of starch in phloem parenchyma cells of infected leaves, 2) callose deposition occurred more frequently in infected midribs. With the use of transmission electron microscopy, it was observed that sieve pores of the midribs of infected plant were plugged with an amorphous substance. Collapse of sieve tubes and companion cells were also observed in HLB-infected midribs but not in healthy midribs. TEM observations also indicated that the HLB bacterium can pass through the sieve plate pore, which suggests that it is unlikely that the HLB bacterium physically caused phloem blockage because multiple bacterial cells were not aggregating. It is likely that the host response results in sieve pore plugging.	Wang	FCPRAC Progress Report (January, 2009).
2009	Microarray analysis (based on 33,879 expressed sequence tag sequences from several citrus species and hybrids) indicated that HLB infection significantly affected expression of 624 genes whose encoded proteins were categorized according to function. The up-regulation of three key starch biosynthetic genes including ADP-glucose pyrophosphorylase, starch synthase, granule-bound starch synthase and starch debranching enzyme likely contributed to accumulation of starch in HLB-affected leaves.	Wang	FCPRAC Progress Report (January, 2009). Co-author of Kim in Phytopathology article (2009)

Appendix L

Witches' Broom Disease Outbreak in Brazil and Control Attempts: Success and Failure in Bahia, Brazil (1989–2009)

Cacao (*Theobroma cacao* L.), is a perennial crop cultivated mainly in small farms in the tropics, particularly Africa, Asia, and Latin America. It is estimated that 5 to 6 million farmers are involved in cocoa production worldwide and 40 to 50 million people depend on cocoa for their livelihood. Loss caused by diseases, which amounts to 30 percent of the total annual crop, is the major limiting factor to cocoa production.

One of the consequences of globalization is the increase in the movement of people and vegetative material between producer countries, a phenomenon that contributes to the spread of plant diseases in a very short time span (Lopes et al., 2007). One such disease is witches' broom in cacao. The witches' broom pathogen, *Moniliophthora* [*Crinipellis*] *perniciosa*, co-evolved with cacao in the diversity center of the Amazon Basin, in South America, and from there spread initially to the neighboring regions and more recently has been spreading to producing areas at farther distance. The first detection of the disease occurred in Surinam, in 1895 (Stahel, 1915), and in the next few decades years reached Bolivia, Colombia, Ecuador, The Guyanas, Grenada, Peru, Venezuela, and Trinidad and Tobago.

In 1989, witches' broom was introduced in Bahia, the major cocoa producing region of Brazil. The disease consumed approximately 300,000 hectares of cacao in Bahia within three years. Losses as high as 100 percent of the crop were observed in many farms and the country lost 75 percent of its annual production, passing from 450 to less than 100 thousand metric tons per year and from an important exporter to importer of cocoa beans. As a result, many farms were abandoned and more than 200 thousand workers lost their jobs, resulting in an intensive migration from rural to urban areas. The cities in the region, extremely dependent on the cacao economy, were not prepared for that massive migration. They faced complex social problems for years after the introduction of the disease. The culture of cacao in Bahia was changed inextricably forever.

Strategies of Control

- The first measure of control attempted was the eradication of the disease by eliminating and burning plants in the focus area (Pereira et al., 1996). However, while the first focus was being eradicated, another one was detected around 100 km apart from the first outbreak and so this measure was determined to be totally ineffective.

- The second measure of control attempted was the use of fungicides and biological control agents. Both of them, despite the encouraging results in preliminary trials, did not result in an effective way of controlling the disease.

- The third measure, which is most widely used in producer countries having the disease and attempted in Brazil, was the phytosanitary pruning. This is quite efficient when the outbreak of this fungal disease severity is low. However, in the first years of the disease in Bahia, the climate conditions to the disease development and the frequent flushing of the plant, resulted in high severity, many plants having hundreds of infected branches (brooms). Despite its efficiency, many farmers stopped doing it because of the high cost involved, aggravating the situation for neighboring farms that took total removal of brooms as their only hope to contain the disease. With the use of more resistant varieties of cacao (as cited below), the number of brooms per plant was reduced and the local epidemiological studies pointed to more regular periods of pruning. This measure of control became widely used in the region but has not proved sufficient to remove the massive disease pressure of the inoculums.

- The fourth measure of control was the use of resistant germplasm; some of them were introduced or developed in the region a long time before the introduction of the disease in the region. However, these varieties despite resistance to witches' broom, were either susceptible to other diseases introduced in the region after the witches' broom (Ceratocystis wilt) or did not reach the levels of yield expected. Recently, new clones were developed and have been released to farmers, without those limitations. Today, around 150 thousand hectares of susceptible varieties were replaced by resistant ones. While this has shown some promise, over the last 20 years there has been little or no progress towards recovery in Bahia.

Lastly, with the overwhelming impact of the disease in Bahia, there is a very serious concern that witches' broom potential to escape into areas where new planting of cacao has begun. The situation today is as dire as it was in 1989!

Appendix M

Vector-borne Maize Pathogens: Lessons Learned

In the first half of the 20th Century, the US Corn Belt escaped serious damage from vector-borne maize pathogens. This was not the case in other parts of the world, i.e. the Mediterranean region (maize rough dwarf), Africa (maize streak), or Latin America (corn stunt). This situation changed when new and rapidly spreading virus-like diseases appeared in the southern fringe of the Corn Belt in the early 1960s.

The region was ill prepared to deal with the problem; few scientists in the Corn Belt were experienced with vector borne plant pathogens. The Ohio State University (OSU) took the lead, hiring faculty with expertise in electron microscopy (1964), plant virology (1966) and arthropod transmission of plant pathogens (1966). Faculty members were stationed at the Ohio Agricultural Research and Development Center, OSU's agricultural research campus in Wooster. The US Department of Agriculture-Agricultural Research Service (USDA-ARS) soon followed suit by adding three scientists, a plant pathologist (1967) and an entomologist (1967) to focus on disease epidemiology and a viral biochemist to add expertise in the emerging field of molecular biology. Later (1980), OSU added a faculty member specializing in plant disease modeling. These scientists joined forces with a veteran maize pathologist and two established corn breeders, one each from OSU and the USDA, on the Wooster campus. Excluding the cost of major equipment purchases (e.g. electron microscopes, ultra-centrifuges, etc.) the annual budget to support the maize virus program in 1968 was ca. $100,000 each for OSU and the USDA.

This cadre of Wooster-based OSU/USDA scientists (maize virus team) collaborated with plant pathologists, entomologists, and plant breeders from other Corn Belt states as well as maize specialists from the southeastern United States who reported similar disease problems. These university and federal scientists met yearly with representatives from the major seed corn companies and other agribusinesses under the authority of the S-70 regional project to share data and plan collaborative research.

The aphid-transmitted maize dwarf mosaic virus (MDMV), a strain of sugar cane mosaic virus, was isolated in early investigations (1964) and thought to be the main cause of stunting disease in the Corn Belt. However, the maize virus team could not consistently associate MDMV with maize stunting disease in the region. Something was missing; a search for additional pathogens continued! Data from greenhouse and field studies, electron microscopy, and insect vector studies ultimately led to the isolation and characterization of maize chlorotic dwarf virus (MCDV) in 1971. It took four years to uncover this unusual, semi-persistently-transmitted, leafhopper-borne virus.

Maize chlorotic dwarf virus along with MDMV proved to be the primary causes of maize stunting disease, not only in the Corn Belt, but in the southeastern US as well. The distribution of MCDV overlaps that of its native vector, *Graminella nigrifrons*, and its over-wintering host, the introduced, perennial weed, johnsongrass. Johnsongrass also is the primary alternate host for MDMV. Fortunately, the northerly distribution of johnsongrass is limited to the southern fringe of the Corn Belt; the leafhopper and aphid vectors migrate to as far north as Canada. Neither virus has ever spread to the heart of the Corn Belt. Control of MCDV and MDMV has been principally by the development of virus tolerant and or resistant varieties and management of johnsongrass. These control measures proved timely and highly successful in returning maize in infested regions to pre-disease production levels.

Spin-off from the formation of the maize virus team in Wooster and its collaboration with workers from more than 20 states has been multifold. Among the discoveries by the team in its first decade was uncovering another complex of corn stunting pathogens endemic to Texas, Florida, and other Gulf Coast states. Causal agents are the corn stunt spiroplasma, the maize bushy stunt phytoplasma, and the maize rayado fino virus. All three are transmitted by the corn leafhopper, *Dalbulus maidis*. Also discovered were the maize mosaic and the maize stripe viruses in these same Gulf Coast States; both are obligately transmitted by the corn delphacid, *Peregrinus maidis*. The beetle vector of the maize chlorotic mottle virus, a virus introduced into Nebraska and Kansas from the Andes was also discovered. So too were the eriophyid mite-borne wheat streak mosaic and wheat spot mosaic viruses in maize as well as the mite-induced phytotoxin, the cause of kernel red streak disease in the Great Lakes region. Information derived from the etiology of these maize pathogens provided researchers elsewhere (in the United States and internationally) with new and better tools to detect and manage maize diseases.

The OSU/USDA maize virus team on the Wooster campus remains as the foremost center, both nationally and internationally, for the study of maize virus diseases. There is a new generation of scientists who have replaced retired members of the original team. These scientists have brought new skills, especially in the molecular arena, to bear upon the discovery, characterization, and management of vector-borne maize pathogens. Dozens of graduate students, post doctoral scientists, visiting faculty and other researchers have been educated, trained or have advanced their careers working with the maize virus team. It should be noted that several "alumni" are now investigating vector-borne citrus pathogens, including HLB.

What lessons learned from the experiences of the maize virus team is relevant to the strategies for the study of HLB? Many factors contributed to the successes of the maize virus team but the following were most critical.

1. The team was formed by recruiting young scientists who were trained in the varied disciplines required for solving the problems associated with vector-borne plant pathogens. Just as important was that they joined forces with experienced faculty familiar with maize diseases and corn breeding in the Corn Belt states.

2. Members of the team were committed to a common goal. All were located on the same campus, many in the same suite of offices and research laboratories. The OSU and USDA scientists, their staff, and the students interacted daily, freely exchanging information and utilizing each other's equipment and resources. Team members formed and, as circumstances dictated, reformed flexible units of two or more specialists to tackle specific problems. Team members met frequently with regularly scheduled meetings and seminars.

Success in solving HLB and other vector-borne pathogens in citrus is most likely to be achieved if a team of scientists, with the relevant skills, tools, and experiences, engage one another and willingly work toward agreed upon goals. Scientists with requisite expertise likely now are in place and conducting HLB research, but they are scattered among a number of institutions and locations. It may not be possible to house key scientists of an HLB team in one laboratory or campus setting. The question then is how to form a virtual laboratory where those tackling HLB can best take on the advantages of working together as a team.