Final Report

Title:	**Rear and Release Psyllids as Biological Control Agents - An Economical and Feasible Mid-Term Solution for Huanglongbing (HLB) Disease**

Sponsoring Agency	NIFA	**Project Status**	COMPLETE
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Program Name: Specialty Crop Research Initiative

Project Director

Thomas Turpen

469-371-2608

catp@citrusrdf.org

Recipient Organization

CITRUS RESEARCH AND DEVELOPMENT

700 EXPERIMENT STA RD

Lake Alfred, FL 338502243

DUNS No. 961745697

Performing Department

{NO DATA ENTERED}

Co-Project Directors	**Departments**
Shatters, Robert	Subtropical Insects Research
Burns, Jacqueline	Office of the Dean for Researc
Polek, Mary Lou	{NO DATA ENTERED}
Browning, Harold	Agricultural Research Service
Patt, Joseph	
Pelz-Stelinski, Kirsten	

Non-Technical Summary

This proposal presents research targeting the elimination of Huanglongbing (HLB) as an economic threat to US citrus production by blocking the ability of the psyllid insect to move the causative agent of this disease between infected and healthy trees. The primary long term goal of this project is to interfere with the spread of HLB within citrus orchards where HLB disease is established and to interfere with the invasion of disease organism into areas where the insect that transmits the causal agent is established, but in which HLB has not been detected, by strategically releasing a nuPsyllid population that is incapable of moving the disease. A further goal is to ensure the necessary adoption of the method by the social system of growers, and understanding and acceptance by consumers and the general public. Once established, this novel system of biological control would be operationally transferred to the citrus industries of U.S states (Florida, California, Texas and Arizona). Other ongoing support, if necessary, will be provided by the stakeholder organizations. We believe current management practices are not sustainable, and in any event psyllid vector eradication has never been achieved, except on small islands. Alternative HLB-management approaches must be developed as a mid-term solution to the HLB problem. Without control measures in hand, citrus growers have no incentive to replace infected trees or to replant entire orchards. The uncertainties associated with HLB will undermine the stability of the industry in currently HLB-free areas. A mid-term solution is crucial to maintain a profitable industry until citrus varieties with resistance to HLB can be developed and released. Therefore, we present a novel and more environmentally friendly alternative strategy, which we will convey to growers and the public. Grower response to this disease has resulted in a mix of increased costs, modifications to long-successful production management systems and acceptance of at least short-term yield and/or quality reductions. Total orchard loss is increasing as HLB spreads. The current situation suggests that without development of an adequate control strategy, commercial citrus production will become economically unfeasible. This disease also is impacting the millions of citrus trees grown in homeowner yards.

Accomplishments

Major goals of the project

The primary long term goal of this project is to interfere with the spread of HLB within groves where HLB is endemic and to interfere with the invasion of CLas into areas where ACP is established but HLB has not been detected. Once released and established, the nuPsyllid population will naturally penetrate and displace the wtPysllid population. A further goal of this proposal is to ensure the necessary adoption of the method by the social system of growers, consumers and the general public in citrus states. To achieve these goals, we propose a three-fold approach: 1). Develop a psyllid management strategy based on the development of psyllid populations incapable of transmitting CLas (nuPsyllid) and strategically release the nuPsyllid population to displace current ACP populations that have invaded the US. 2). Provide optimized management strategies for integration of the proposed population displacement technique into current management practices: a. Southeast and Southern U.S. (FL and TX) where both the ACP and CLas are endemic. b.Western U.S. (CA, AZ) where ACP is present and spreading while there is currently no detection of HLB. 3). Integrate the management strategies with monitoring strategies to continually assess effectiveness and provide outreach education to the grower stakeholders and citizens about the control strategy. The feasibility of the approach proposed here is supported by the experience with HLB management in Florida through the creation of Citrus Health Management Areas (CHMAs). The CHMA is based on the recognition that HLB has an important edge effect. Although insecticide applications can control ACP populations within the grove, without effective ACP management in the surrounding areas, CLas-bearing ACP rapidly returns. A CHMA coordinates the insecticide sprays and other management activities over a large area, thus greatly reducing the edge effect of ACP re-invasion. Thus, we are proposing that most nuPsyllid releases will be focused at the periphery of CHMAs and other smaller management areas to displace the endemic population at the periphery. Vigorous ACP control measures would be continued temporarily in the interior of the management area but would be gradually tuned down to allow populations of CLas-transmission-deficient ACP populations to become established

What was accomplished under these goals?

Our first objective was to develop modified ACP colonies (nuPsyllid) that could be reared and released to replace the wild populations and that would be unable to move CLas in the environment. It is not possible to rely on selection alone to spread this trait into a wild population of psyllids because the inability to spread disease would itself offer no survival advantage. Success with this approach requires a non-Mendelian method to drive the genes into the population, a Driver System. Six psyllid viruses and one psyllid bacteria were described in this work that could be adapted for this purpose. Ideally, the inability to spread disease would be a trait encoded in the chromosomal DNA of the insect. We demonstrated two generally applicable methods to drive a trait into an insect population from components assembled into the genome. In the first case, population replacement is achieved only if the population is released above a dominant numerical threshold. For example, if the 60% or greater of the released population is the desired nuPsyllid type (incapable of spreading disease) in 10 generations it would locally replace essential all of the wild psyllids. Whereas, if 40% or less of the released population was the nuPsyllid type, then the wild type would predominate and the nuPsyllid population would disappear. This is a very attractive feature of gene drive because it is essentially reversible. In the second case, population replacement is achieved with release of a nuPsyllid population that continues to spread in the population. This is desirable for citrus disease management because of the large number of trees that occupy both the urban and commercial lands. Initial assessments have not identified the required variation in CLas transmission to occur naturally in ACP populations. The effector is the content of the phenotypic change we aim to introduce, an Effector Mechanism. Candidate effectors have been identified through multiple parallel methods of investigation including bioinformatics, proteomics, yeast two-hybrid (Y2H), peptide-ligand and antibody fragment scFV-ligand libraries. Experimental results and theory based on models of CLas movement within ACP have guided selection of priority effectors for future studies. This work also provides a detailed molecular description of how the CLas bacterium becomes established in and moves from the gut of the ACP, into the body of the insect and then the salivary gland. Several genes were identified that block the ability of the insect to spread disease when they are disrupted. Data is made available publically through at the website (www.sohomoptera.org/ACPPoP).
Our second objective was to provide optimized management strategies for integration of the proposed population displacement technique into current management practices. Detailed models of the conditions for dissemination of psyllids and the adoption of this technology by society were established. Our team improved the ability to rear, release and monitor psyllids in detailed studies on the composition artificial media, shipping conditions, attractants and improved traps designs. Analytical infrastructure was developed to coordinate interaction between technologists and social scientists and to perform prospective analysis of the probable uptake of technology and its impact if deployed. The agent-based model of ACP dynamics is being used to find optimal disease management approaches, while the ACP phenology model is being used to evaluate the effectiveness of its ACP suppression program by providing a simulated baseline of development rates against which field observations in areas with active ACP control can be compared. A new economic surplus model developed for the work evaluates the likely financial outcome of deployment of gene drive technology and reveals that low deployment, low benefit equilibria may exist for such technologies under plausible assumptions about knowledge spillover effects among

technology developers. We embedded the somewhat technical elements of the model formulation in a more accessible systems analysis approach that also yielded useful qualitative insights into factors that are likely to determine the sustainable development of this, or similar, technologies. These are generically useful methodological developments for the analysis of technology development and adoption in agriculture.
For the third objective, the project's outreach plan was developed to help the public understand the release of a modified psyllid. Because a modified psyllid was not developed during the project period, our outreach emphasis shifted to developing information that would help growers and the general public better understand the techniques that are being used by scientists to attempt to develop a nuPsyllid population, the importance of genetic engineering, and the other tactics that scientists are using to solve the Huanglongbing problem. It is an important principle in establishing and maintaining trust between scientists and the general public that technology solutions under investigation and development are communicated as they are developed. The web site, "Science for Citrus Health" was developed as a UC ANR web site and it went live in May 2015, http://ucanr.edu/sites/scienceforcitrushealth/. The web site describes the ACP/HLB situation and provides resources for growers to better understand the techniques that are being developed to battle the disease. Postcards announcing the web site have been distributed to citrus growers at meetings in California and Florida. A poster presentation was made for the International Research Conference on HLB in Orlando in 2017, highlighting resources available on the Science for Citrus Health Web pages. The web site has a section called research snapshots that describe approaches that are currently being pursued nationwide to manage HLB (both engineered and non-engineered approaches). The team worked with researchers to write up descriptions of their research strategies and accomplishments and we then translate that material into language that can be readily understood by general readers. These Research Snapshots are divided into four categories, Early Detection Techniques (3 entries), Established Orchard solutions (5 entries), Replant solutions (2 entries) and nuPsyllid (3 entries). Each of the snapshots is written in a form that can be used as a fact sheet to be passed out at extension events including using insect viruses to combat the psyllid and stopping spread of CLas using beneficial bacteria. The Science for Citrus Health blog alerts the citrus industry and the media to updates of the web site. The web site is utilized by media to showcase research activities and helps communicate the progress being made in the fight against Huanglongbing to growers and the general public. The site is developing twitter followers as well. The outreach team completed the fact sheet titled 'What makes lemons, oranges and limes look and taste different?' The fact sheet defines crop and insect genetic engineering in the context of citrus and includes the nuPsyllid project as a portion of the examples. The fact sheet is available on the web site and is used as a handout at grower meetings. An extensive collection of PowerPoint slides, covering a diverse array of topics, is posted on the web site and updated as new information comes out. The PowerPoint is used by extension and research personnel to educate growers about the approaches researchers are using to combat HLB. A grower seminar "Food fights in the marketplace: is there a way to use genetics to address HLB disease in citrus?" was developed and presented in several locations. The seminar covers explanations of how plants and insects are genetically modified, consumer attitudes towards GM, and potential uses of GM for the citrus industry. Grower preferences and opinions were surveyed in polls at industry conferences.

What opportunities for training and professional development has the project provided?

Sub-project elements of this project continue in University and USDA laboratories in a number of states, providing considerable professional training to undergraduate and graduate students through direct involvement in the nuPsyllid project. The project employed a number of Post-Doctoral trainees in the labs, whose contributions to the research objectives provided them additional professional training. All involved in this project were exposed to the approaches and mechanics of team research on a large scale, with team meetings that outlined how the component research objectives fit into the larger picture. The project put the Quantitative Biology & Epidemiology (QBE) lab at UC Davis in the center of some of the most difficult issues facing US citrus industries in the struggle against ACP/HLB. QBE's role in the project raised our profile as an interdisciplinary research team resulting in an almost continuous series of invitations to perform similar roles for other teams making applications to CDRE and to other NIFA programs. Considering just CDRE, as an example we are involved in two proposals for the current round of funding, where our role will (if the projects are supported) be essentially to coordinate interaction between technologists and social scientists and to perform prospective analysis of the probable uptake of technology and its impact if deployed. QBE's capacity to act as an effective coordinating hub for projects involving technology and social science has led to receiving close to $2M in MAC funding to coordinate research on Early Detection Technologies and on ACP sampling and population dynamics in CA, and to playing a coordinating role in industry-funded research and outreach in California; this is all built on the ground-work done in the nupsyllid project.
In addition to the direct research funded by the nuPsyllid grant, D. citri viruses identified under the nuPsyllid project allowed for additional and expanded research opportunities towards HLB and D. citri. Dr. Bryce Falk (UC Davis) and William Dawson (Univ. of Florida, CREC), PIs, and eight Co-PIs were awarded a 3-year $4.7 million grant from USDA NIFA SCRI. This grant entitled "Non-transgenic, near term RNA interference-based application strategies for managing Diaphorina citri and citrus greening/Huanglongbing (HLB)", and has the option for 2 additional years of funding at $2.7 million. This grant was only possible because of our research successes demonstrated from the nuPsyllid grant and CRDF funded research by Dawson. This project will build on successes and expand efforts for using D. citri and citrus viruses to induce desirable RNAi effects in

D. citri, focusing the effort to use other D. citri viruses discovered in the original virus discovery part of the nuPsyllid grant.
At the Univ. of Arizona Dr. Brown's lab's involvement in Nu-Psyllid led to cooperation between the Brown lab and the Arizona Department of Agriculture to establish HLB qPCR diagnostics and assist with detection of the bacterium in citrus leaf and psyllid samples collected in the Arizona survey/quarantine effort. Subsequently, overflow psyllid samples are now being processed and analyzed for CLas presence by qPCR. The lab is certified annually by APHIS-PPQ.
As a result of Dr. Shatters' USDA lab finding that learned interdiction peptides identified to kill Liberibacter in citrus would not work in the direct application of creating a 'nuPsyllid', other strategies for use of these peptides in controlling HLB are being evaluated as part of two subsequent NIFA SCRI grants:
a. As part of the UC-Davis NIFA SCRI grant (Non-transgenic, near-term RNA interference-based application strategies for managing Diaphorina citri and citrus greening/Huanglongbing (HLB) the use of these peptides in conjunction with RNAi inducing dsRNA molecules that target essential psyllid genes. The idea here is that by combining control strategies wit very different modes of action we will develop robust psyllid/HLB controls method. Delivery via nuclear transformation of citrus and via expression in the Citrus tristeza virus vector are being evaluated
b. Evaluation of direct delivery of these peptides to the citrus using injection and novel delivery strategies as part of the KSU NIFA SCRI grant (Developing an infrastructure and product test pipeline to deliver novel therapies for citrus greening).
Additional benefits from the project have enhanced other collaborative funding prospects and involvement with HLB community: (1) New collaborative basis with certain team members (Kirsten, Nabil). (2) Psyllid Omics data will be made available to the ACP omics-databases (after publishing key papers); ACP transcript mapping to ACP genome. (3) Genome sequence drafts for endosymbionts/dissected ovaries (ACP). (4) Expansion beyond nuPsyllid: (a.) Arizona Dept of Agriculture and California Dept. of Food and Agriculture: qPCR monitoring of psyllids for CLas; for AZ-Dept. Agric. qPCR monitoring of citrus trees; certified testers 4 years+. (b.) Consortium participant: HLB Data Analytic Tactical Operations Cell (DATOC). Survey data, risk models, early detection Technology (EDT) and other data sources are available to inform decision makers on management and mitigation of the spread of HLB. It is imperative to establish an analytic group to process incoming information and intelligence, promote cross-analysis and comparison of diverse data sources and utilize our diverse expertise as the epidemic intensifies. This group will seek to use sound science and other data-based information streams to inform planning as well as operational strategies and logistics in a cross disciplinary environment. The group will meet virtually or in person to exchange analytic approaches and datasets, when feasible, to collaborate in making tactical recommendations to regulatory, operational and organization decision makers on tactics to mitigate HLB. The focus will be citrus in California, but also potentially other locations. The California Citrus Research Board is funding this project to provide dedicated staff, IT infrastructure and regular meetings (travel to in-person or virtual meetings). The objective of these meetings is to provide a forum where experts can combine their aggregated expertise to address complex questions from regulators, industry and other citrus stakeholders in mitigating the threat of Huanglongbing.
The project established important pieces of modeling infrastructure that are continuing to be used in on-going research and in providing tactical support to disease management programs in Florida and California. The agent-based model of ACP dynamics, developed in the collaboration between UC Davis and USDA-ARS is being used to find optimal disease management approaches, while the ACP phenology model developed by OSU Corvallis in collaboration with UC Davis is helping the CPDPC in California evaluate the effectiveness of its ACP suppression program by providing a simulated baseline of development rates against which field observations in areas with active ACP control can be compared.
The ability to transform insect pests of agriculture such as psyllids, will enable gene drive approaches to population eradication, replacement and suppression. It will also facilitate the emerging commercial applications of sterile insect technologies based on genetics. The production and release of sterile males by biotechnology is less costly and more effective than a population approach based on irradiation. There is a high cost of production because of the expense of the radiation source and centralized production and the insects released will be much less fit than those created by biotechnology methods. This method has proved effective in some cases such as the medfly and screwworm. A more efficient, decentralized, low-capital, biotechnology approach should make this population suppression approach applicable to additional insect pests.

How have the results been disseminated to communities of interest?

Annual Team meetings of stakeholders, administrative management and advisors as well as numerous meetings of citrus growers in California, Texas and Florida at which presentations on project goals & objectives were presented.
Psyllid bioinformatics database - major accomplishment from Dr. Brown's (UAZ) objective - six annotated transcriptome datasets that can be mined to identify candidate effectors, design primers for validation and qPCR quantification (of gene expression), and select dsRNA targets for RNA interference knockdown experiments. Publicly available (www.sohomoptera.org/ACPPoP ; Vyas et al. 2014, Fisher et al. 2015); additional databases to be released upon publication of other submitted manuscripts.
Important pieces of modeling infrastructure continue to be used in on-going research and provide tactical support to disease management programs in FL and CA using the agent-based model of ACP dynamics, developed in collaboration between UC Davis & USDA-ARS to find optimal disease management approaches, while the ACP phenology model developed by OSU

Corvallis in collaboration with UC Davis is helping the CPDPC in CA evaluate effectiveness of its ACP suppression program by providing a simulated baseline of development rates against which field observations in areas with active ACP control can be compared.
Collaboration between UC Davis & UW Madison has yielded an economic surplus model for evaluating the likely financial outcome of deployment of gene drive technology, revealing that low deployment & low benefit equilibria may exist for such technologies, which are generically useful methodological developments for the analysis of technology development and adoption in agriculture.
Peggy LeMaux, molecular biology specialist at UC Berkeley, developed a grower seminar "Food fights in the marketplace: is there a way to use genetics to address HLB disease in citrus?" The seminar was presented in several locations in CA as part of the Citrus Research Board Grower Seminar series, and covered explanations of how plants and insects are genetically modified, consumer attitudes towards GM, and potential uses of GM for the citrus industry.
A survey was conducted at the Florida Citrus Expo in August of 2016 with the results indicating that although 93% of the respondents practiced what they considered to be "conventional" grove management practices, 46% would definitely accept GMO alternative technologies to solve HLB; another 44% stated they would likely be in favor of accepting such solutions if they were available. Eighty per cent felt the greatest hurdle to overcome in adoption of GMO crops was public perception; 60% favored a technology that would target trees (i.e. HLB-resistant varieties by genetic modification).
The web site, **"Science for Citrus Health"** developed by Peggy Lemaux (UCR) and collaborator Lukasz Stelinski (UF) went live in May 2015, http://ucanr.edu/sites/scienceforcitrushealth/ and has had over 2000 visits to date. The web site describes the ACP/HLB situation and provides resources for growers to better understand the techniques that are being developed to battle the disease. A poster presentation was made for the International Research Conference on HLB in Orlando in 2017, highlighting resources available on the Science for Citrus Health Web pages. Funding has been obtained from other sources to continue the web site after the nuPsyllid project funding ends.
The web site has a section called research snapshots that describe approaches currently being studied nationwide to manage HLB (both engineered and non-engineered approaches) in lay terms, and contains downloadable fact sheets for distributing at extension events. These Research Snapshots are divided into four categories: Early Detection Techniques, Established Orchard solutions, Replant solutions and nuPsyllid.
An extensive collection of PowerPoint slides covering a diverse array of topics, is posted on the web site and updated as new information comes out. To date there have been nearly 100 downloads from the site. Topics included in the customizable slide set include descriptions of genetic engineering of plants and insects and how these might be used to address HLB, nonengineering approaches, regulatory issues, consumer attitudes, labeling considerations and trade issues. The powerpoint is used by extension and research personnel to educate growers about the approaches researchers are using to combat HLB.
The Public Outreach effort has become a hub of communication about citrus technologies for both grower and the general public, critical to building trust that technologies, especially those supported with public funding, are not perceived to have been developed in secret, an important principle in establishing and maintaining trust between scientists and the general public that technology solutions under investigation and development are communicated as they are developed.
The outreach team compiled a fact sheet titled 'What makes lemons, oranges and limes look and taste different?', defining crop and insect genetic engineering in the context of citrus and includes the nuPsyllid project as a portion of the examples; it is available on the web site and is used as a handout at grower meetings.
The **Science for Citrus Health blog** alerts the citrus industry and the media of updates to the web site, also utilized by media to showcase research activities and helps communicate the progress being made in the fight against Huanglongbing to growers and the general public. The site is developing twitter followers as well.
The following references are IN ADDITION to Presentations listed under the Publications made by J Brown, F Dong, BW Falk, PD Mitchell, S. Nouri in Brazil, France, Hawaii, Mexico, Spain and the United States:
Nouri, S. Diverse array of new viral sequences identified in worldwide populations of the Asian citrus psyllid (Diaphorina citri) using viral metagenomics. Society for Invertebrate Pathology, July 27, 2016, Tours, France.
Green blog - http://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=24671&sharing=yes
Tim Hearnden Capital Press. New UC website explains scientific advances on citrus psyllid,
HLB http://www.capitalpress.com/Research/20170726/new-uc-website-explains-scientific-advances-on-citrus-psyllid-hlb
California Citrus Mutual https://www.cacitrusmutual.com/just-facts-maam-uc-launches-readers-digest-website-share-important-research-updates-citrus-growers/
AgAlert, California Agricultural Newspaper http://agalert.com/story/?id=11043
FreshPlaza http://www.freshplaza.com/article/179124/New-website-simplifies-latest-citrus-greening-research-for-farmers
ANR Report blog - http://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=24865
Pests in the Urban Landscape blog - http://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=24818
Retail and Garden Center IPM news http://ipm.ucanr.edu/PDF/PUBS/Summer_2017_Retail_Newsletter.pdf
http://campaign.r20.constantcontact.com/render?m=1102549393270&ca=35747d32-97cc-416e-bf20-5b722072ef32

What do you plan to do during the next reporting period to accomplish the goals?

{Nothing to report}

Participants

Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	4.3	0	1	5.7	11
Professional	0.5	0	0	0	0.5
Technical	5.6	0	0	0	5.6
Administrative	0.3	0	0	0	0.3
Other	0	0	0	0	0
Computed Total	10.7	0	1	5.7	17.4

Student Count by Classification of Instructional Programs (CIP) Code

Undergraduate	Graduate	Post-Doctorate	CIP Code
	1	3	26.05 Microbiological Sciences and Immunology.
	1	3	26.09 Physiology, Pathology and Related Sciences.

Target Audience

Target audiences include the primary benefactors of the research, the U.S. citrus growers. In addition, the target is the scientific community who is engaged in developmental research that has allowed this research project to be envisioned, and on whose progress we will continue to move forward. The general public is a target of our outreach. As consumers, they are interested and concerned about how research solutions are implemented to solve practical problems, and have shown interest in the foundations of this research project. Finally, policy-makers who often are involved in funding research for Florida citrus, need to be appraised of the project, its goals, and expectations that come from progress. The Outreach Team has determined that 1) because the effector and driver systems are all progressing equally it will not be possible to eliminate one or more from the outreach efforts and 2) an educational program should focus on the context of genetic technologies in general so that the nuPsyllid option for disease management is contrasted for example with a genetically modified citrus host and other technology options. Together with molecular biologist and extension expert Peggy Lemaux, Powerpoint presentation materials based on 3 grower interview events in California have been drafted, adapted as handouts and incorporated into the website "Science for Citrus Health."

Products

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2015	YES

Citation

Yuan, Qing, Jordan, Ramon, Brlansky, Ronald H., Minenkova, Olga, Hartung, John. 2015. Development of single chain variable fragment (scFv) antibodies against Xylella fastidiosa subsp. pauca by phage display. J. Microbiological Methods 117:148-154. http://dx.doi.org/10.1016/j.mimet.2015.07.020.

Accession No. 230893 **Project No. FLAW-2012-01527**

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2015	YES

Citation

Fang Ding, YongPing Duan, Cristina Paul, Ronald H. Brlansky and John S. Hartung 2015. Localization and distribution of ‘Candidatus Liberibacter asiaticus’ in citrus and periwinkle by direct tissue blot immuno assay with an anti-OmpA polyclonal antibody. PLoS One: 10(5):e0123939.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2016	YES

Citation

Yuan, Qing, Jordan, Ramon, Brlansky, Ronald H., Minenkova, Olga, Hartung, John. 2016. Development of single chain variable fragment (scFv) antibodies against surface proteins of ‘Ca. Liberibacter asiaticus’. J. Microbiological Methods. 122:1-7. http://dx.doi.org/10.1016/j.mimet.2015.12.015.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2016	YES

Citation

Fang Ding, Yongping Duan, Qing Yuan, Jonathan Shao and John S. Hartung. 2016. Serological detection of ‘Candidatus Liberibacter asiaticus’ in citrus and the identification of a promising secreted chaperone protein responding to cellular pathogens. Scientific Reports 6:29272. DOI: 10.1038/srep29272.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2017	YES

Citation

Huawei Liu, Sagheer Atta, and John S. Hartung. 2017. Characterization and purification of proteins of used for the production of antibodies against ‘Ca. Liberibacter asiaticus’. Protein Expression and Purification 139: 36-42

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2017	YES

Citation

Fang Ding, Cristina Paul, Ronald H. Brlansky and John S. Hartung 2017. Anti-Omp A polyclonal Antibody-based in Situ Immuno Tissue Print and Immune Capture-PCR, Diagnosis and Detection of Candidatus Liberibacter asiaticus. Scientific Reports 7:46467. DOI:10.1038/srep46467

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2016	YES

Citation

Stover, E., Hall, D.G., Shatters, R.G. and Moore, G.A., 2016. Influence of citrus source and test genotypes on inoculations with Candidatus Liberibacter asiaticus. HortScience, 51(7), pp.805-809.

Type	Status	Year Published	NIFA Support Acknowledged
Book Chapters	Published	2016	YES

Citation

Brown, J.K., Cicero, J.M., and Fisher, T.J. 2016. Psyllid-transmitted Candidatus Liberibacter species infecting citrus and solanaceous hosts. Pages 399-422 in: Vector-Mediated Transmission of Plant Pathogens, (ed.) Brown, J.K. American Phytopathological Society Press, St. Paul, MN. 496 pp.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2015	YES

Citation

Cicero, J.M., Stansly, P.A., and Brown, J.K. 2015. Functional anatomy of the oral region of the potato psyllid (Hemiptera: Triozidae) Ann. Entomol. Soc. Am. 108: 743-761.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2014	YES

Citation

Soderlund, C.A., Nelson W.M., and Goff, S.A. 2014. Allele Workbench: Transcriptome pipeline and interactive graphics for allele-specific expression. PLoS ONE 9(12): e115740. doi:10.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Other	2017	YES

Citation

Brown, J.K., Saberi, E., Brown, C.C., and Rast, TJ. 201x. Gene expression AAP time course.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Other	2017	YES

Citation

Fisher, T.W., Rast, T.J., Soderlund, C., He, R, Gang, D. R. and Brown, J.K. 201x. Interactions between ‘Ca. Liberibacter’ effector-psyllid proteins, and a model for systemic invasion of the psyllid host and vector. Parasite and Vector (in prep).

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2013	YES

Citation

Cicero, J.C., and Brown, J.K. 2013. SEM- and TEM-Informed Anatomical Observations of Ca. Liberibacter parasite localization in its psyllid host. Third International Research Conference on Huanglongbing-IRCHLB III. Orlando, Florida, February 4-7, 2013 (poster).

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2013	YES

Citation

Fisher, T., Cicero, J.M., and Brown, J.K. 2013. Connecting anatomical and molecular data of Candidatus Liberibacter asiaticus and solanacearum during vector-mediated transmission. Third International Research Conference on Huanglongbing-IRCHLB III. Orlando, Florida, February 4-7, 2013.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2013	YES

Citation

Fisher, T., He, R., Soderlund, C., Smith, W., Vyas, M., Gang, D., and Brown, J.K. 2013. A comparative transcriptomics approach to elucidate psyllid-Ca. Liberibacter interactions. Third International Research Conference on Huanglongbing-IRCHLB III. Orlando, FL, February 4-7, 2013.

Accession No. 230893 **Project No. FLAW-2012-01527**

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2013	YES

Citation

Fisher, T., Nelson, W., Vyas, M., He, R., Willer, M., Gang, D.R., Soderlund, C., and Brown, J.K. 2013. Publicly available website for the identification of psyllid-'Ca. Liberibacter interactors' using comparative transcriptome analysis. Phytopathol. 103(6S): 44. APS-MSA meeting, August 8-14, 2013. Austin, TX

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2015	YES

Citation

Brown, J.K. 2015. Assessment of detection methods and the threat of Candidatus Liberibacter in seed and other propagative plant materials. APS Symposium presentation, Pasadena, CA Aug 1-5, 2015.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2015	YES

Citation

Brown, J.K., Rast, T.J., and Fisher, T.W. 2015. Transcriptomics, proteomics, and yeast-2-hybrid analyses reveal genes pathways important for Candidatus Liberibacter sp. circulative, propagative transmission. International Research Conference on HLB, Caribe Royale, Orlando, FL February 9-13, 2015 (oral presentation).

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2015	YES

Citation

Fisher, T.W., and Brown, J.K. 2015. Optimization of dsRNA knockdown assays to evaluate RNAi efficacy for gene silencing in the potato psyllid. International Research Conference on HLB, Caribe Royale, Orlando, FL February 9-13, 2015 (oral presentation).

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2016	YES

Citation

Brown, J.K., Rast, T.J., Cicero, J.E., and Fisher, T.W. 2016. Proposed model for Candidatus Liberibacter asiaticus and solanacearum systemic invasion and multiplication in the psyllid host and vector. American Phytopathological Society, Tampa FL, July 28-Aug. 4, 2016.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2016	YES

Citation

Brown, J.K., Rast, T.J., and Fisher, T.W. 2016. Psyllid vector-Liberibacter interactions at cellular and molecular interfaces. Symposium: Biology, Ecology and Management of the Asian Citrus Psyllid Diaphorina Citri, Vector of Huanglongbing International Congress of Entomology (ICE). Orlando, FL. September 26, 2016.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2017	YES

Citation

He, R., Willis, M., Fisher, T.W., Soderlund, C., Pelz-Stelinski, K., Brown, J.K., and Gang, D.R. 2017. Impact of Candidatus Liberibacter asiaticus infection on Asian citrus psyllid transcriptome. Fifth International Research Conference on Huanglongbing-IRCHLB III. Orlando, FL, March 13-17, 2017.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2017	YES

Citation

Wang, X., R. He, J. Wang, A. Berim, J.-J. Park, T.W. Fisher, J.K. Brown, D. R. Gang. 2017. Combined UPLC-MS/MS and MALDI-MSI analyses identify metabolic differences between CLso-infected and uninfected psyllids. Fifth Intern'l Res. Conf. on HLB. March 14-17, 2017. Orlando, FL (oral).

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2013	YES

Citation

Brown, J.K., Biofilms and yellow dragons. College of Agriculture, Spring Awards Luncheon. Featured Speaker, University of Arizona, Tucson, AZ, March 22, 2013.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2013	YES

Citation

Brown, J.K., Psyllid-Liberibacter complexes: Emerging vector-pathogens. National Plant Disease Recovery System, Arlington, VA, April 15, 2013.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

Citation

Brown, J.K., Invited presentation: Emergent and Invasive Psyllid-Liberibacter complexes. Western Plant Board 95th Annual Conference. Tucson, Arizona. March 24-27, 2014.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

Citation

Brown, J.K., Symposium: Ca. Liberibacter solanacearum: a psyllid-transmitted, endemic and exotic, emergent fastidious prokaryote. Plant Virus-Vector Complexes in the Western US, APS-Pacific Division Meeting. Vector Symposium, Bozeman, MT July 8-11, 2014.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

Citation

Brown, J.K., Invited seminar: Psyllid transcripts with potential involvement in Ca. Liberibacter invasion and propagative transmission: Toward RNAi mediated abatement of citrus greening and zebra chip diseases. Vet-Science Microbiology Seminar, University of Arizona Nov 10, 2014.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2015	YES

Citation

Brown, J.K., Invited Workshop: Citrus greening: biofilms and yellow dragons. USDA-APHIS Workshop, Phoenix AZ. Jan 29, 2015.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2015	YES

Citation

Brown, J.K., Invited Seminar: Application of RNAi to interfere with insect transmission of citrus greening disease. UF Whitney Marine Biosciences Laboratory, Augustine, FL Feb 13, 2015.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2016	YES

Citation

Brown, J.K., Maricopa County Master Gardeners (K. Young, host). Insect vectors of plant and fastidious bacterial pathogens in Arizona. Maricopa Co. Extension Center, Phoenix, AZ. April 20, 2016.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2016	YES

Citation

Brown, J.K., Invited speaker: Pacific Branch-ESA symposium Animal and Plant Vector Biology: Addressing Old Questions with New Technologies. A cumulative model for Liberibacter invasion and circulation based on electron microscopy, functional genomics, proteomics, and yeast-2-hybrid analyses. April 6, 2016. Honolulu, HI. April 2-7, 2016.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2017	YES

Citation

Brown, J.K. 2017. Ca. Liberibacter tactics enabling invasion and circulative-propagative transmission by the psyllid host. US Arid-Land Agricultural Research Center, Maricopa, AZ March 27, 2017.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2017	YES

Citation

Brown, J.K. 2017. Psyllid vector biology, Liberibacter-psyllid interactions. Psyllid-Liberibacter Workshop, Guatemala May 16-18, 2017.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2017	YES

Citation

Brown, J.K., Rast, T.J., Cicero, J.E., and Fisher, T.W. 2017. Psyllid vector-Ca. Liberibacter interactions at cellular and molecular interfaces. Third Hemiptera-Plant Interactions Symp., Madrid, Spain, June 4-8, 2017.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2017	YES

Citation

Kandul, Nikolay, M. Guo, and B. Hay. July 2017. A positive readout for site-specific MRNA cleavage. PeerJ, DOI 10.7717/peerj.3602.

Type	**Status**	**Year Published**	**NIFA Support Acknowledged**
Conference Papers and	Published	2016	YES

Citation

Falk, B. W. 2016. RNAi-based strategies against insect vectors of plant pathogens. Plenary lecture. Sept 20, 2016, International Citrus Congress. Foz do Iguacu, Brazil.

Type	**Status**	**Year Published**	**NIFA Support Acknowledged**
Conference Papers and	Published	2016	YES

Citation

Falk, B. W. 2016. RNAi-based strategies against the Asian citrus psyllid, Diaphorina citri. Fundecitrus, Araraquara, Brazil. Dec. 16, 2016.

Type	**Status**	**Year Published**	**NIFA Support Acknowledged**
Conference Papers and	Published	2017	YES

Citation

Nouri, S. 2017. Applying viral metagenomics to discover new viruses of insect vectors of plant pathogens: the Asian citrus psyllid. APS Annual Meeting Hot Topic. San Antonio, TX, August, 2017.

Other Products

{Nothing to report}

Changes/Problems

Our team recognized from the outset that creating and releasing a psyllid population that was refractory to disease spread would be a high-risk but potentially a high-payoff intervention that was complimentary to other research. To mitigate technical risk, we implemented a strategy that launched parallel research strategies both with respect to manipulating the genotype of the population (Driver Systems) and the phenotype of blocking disease spread (Effector Mechanism). To anticipate downstream regulatory requirements for phased testing, we refined methods of rearing, releasing and monitoring nuPsyllid populations. Central to the phased testing approach to biological release is the need to build, validate and refine models to predict effects in space and time across real landscapes. The fundamental difference in this type of program is the notion of a Driver System, a biotechnological tool to spread a trait in a population. The total genetic structure of a population includes, nuclear-encoded chromosomal genes but also endosymbiont and pathogenic bacteria which in the case of the genus Wolbachia in insects, are inherited almost like the DNA of cytoplasmic organelles. Likewise, any biological population hosts a plurality of viral genomes, including many that induce little or no symptoms. These viral genomes are naturally-occurring sequences that can be manipulated as part of a system of pest management.

The strategy with this concurrent development plan was to be able to adapt our budgets and focus if there was a clear intervention opportunity that became available with any of the three Driver Systems. It was the consensus of the Team Leaders, Advisors and Stakeholders in several interim meetings that each of these approaches had good merit and progress. We enhanced the effort on achieving psyllid transgenesis, obtaining useful insect/psyllid cell lines and on modeling effects of RNAi on a landscape scale that are directly related to the goals of this project. We updated project objectives across all sub-award budgets to optimize the remaining use of funds with the need to provide continuity to other funded programs and to generalize the biotechnology tools, models and outreach goals.

At the end of this research project a robust infrastructure was established for communicating innovations to growers and the general public and in modeling the spread and adoption of nuPsyllid and related technologies. There were no major difficulties encountered with the outreach and socio-economic modeling portions of the project. Likewise, the efforts to rear, release and monitor psyllids in the population developed in this and related work and likely sufficient for the nuPsyllid application. Future improvements in this technical area are likely to reduce costs of an intervention.

The foundational knowledge obtained by both Effector-Mechanism teams has yielded many candidate genes that can be further evaluated in Driver Systems as they become available. There is a comprehensive database of high quality annotated sequences (protein, RNA, DNA) and a detailed molecular description of the complete life-cycle of CLas moving through a psyllid from one citrus site of infection to another. Both of these teams have generated new actives (RNAi and peptides) and small molecule targets for intervention. These are important leads for product development but it is unclear whether there are practical delivery methods except delivery in transgenic trees. This will require further innovation and especially cost reduction before they are available for growers. A major bottleneck in this program, was to develop a high throughput method to rank Effector candidates. The work with RNAi was adequate for the purpose but it would be better to express sequences directly in the Driver system of choice. Therefore, the major emphasis of the project remained on the challenges of finding Drivers that could create the desired nuPsyllid phenotype, refractoriness to spread of HLB.

We set out to survey Wolbachia bacteria within psyllids to look for and/or create combinations that might result in material interference with acquisition or transmission of CLas. However, these bacteria are part of the Florida psyllid population structure and any adequate description of the behavior of the population must include these endosymbionts as part of the system. This description is now completed and has led to new concepts of manipulating endosymbiont interactions to potentially create the refractory phenotype. This line of research is well supported in ongoing funded programs. The ability to transform Wolbachia would be an important advance.

Likewise, the survey of psyllid viruses from global collections has uncovered a rich diversity of viral genomes. These also are part of the endogenous psyllid population dynamics in Florida and elsewhere. Full-length clones have been obtained and several of these viruses are likely to prove useful as tools to introduce genes in to psyllids and understand their function in the search for a refractory phenotype and may well prove useful as novel biocontrol agents for population suppression. This line of research is also well supported in ongoing funded programs but there was not a paratransgenesis system developed in the timeframe of the research funded.

The Driver System based on manipulation of chromosomal DNA is likely to be the most robust and effectively deployed. All the necessary components have been assembled and shown to validate multiple theoretical predictions in model systems. There are versatile variations that provide options for confinement and reversal that may be required for initial use in phased testing. The only barrier to progress here is the lack of transgenesis in the psyllid. This the single most important technical challenge. There is a great need for robust and useful insect and psyllid cell lines for use in all the Driver System research. There should be a major effort to bring a variety of new technologies to focus on insect transformation in general, including psyllids. A breakthrough tool or process here would likely make testing the nuPsyllid concept feasible with various chromosomal driver concepts and existing candidate effectors. The transgenesis system needs to perform with reasonably high frequency to be of practical use.

Early ACP embryos can be micro-injected with foreign DNA with a reasonable level of survival that resulted in nymphal hatching. However, while injected and control non-injected nymphs were able to hatch on leaves, only a few have been able to continue nymphal development, and those surviving to adulthood have accounted for less than 1% of hatched nymphs. Thus, the current state of the ACP transformation project at the termination of the grant funding period is that a foundation of knowledge and methodology has been created that should allow continued efforts in evaluating potential transformants. The major roadblock at this time is the inability of newly laid eggs to survive after transfer from flush for injections, and therefore efforts are necessary to improve viability after removal from flush, potentially using artificial media.

The ability to transform insect pests of agriculture such as psyllids, will enable gene drive approaches to population eradication, replacement and suppression. It will also facilitate the emerging commercial applications of sterile insect technologies based on genetics. The production and release of sterile males by biotechnology is less costly and more effective than a population approach based on irradiation. There is a high cost of production because of the expense of the radiation source and centralized production and the insects released will be much less fit than those created by biotechnology methods. A more efficient, decentralized, low-capital, biotechnology approach should make this population suppression approach applicable to additional insect pests.