2017-18 USDA, NIFA SCRI Citrus Disease Research and Education Program Awards

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| BIOPESTICIDAL DSRNA THERAPY FOR PSYLLID MORTALITY AND ABATEMENT OF VECTOR-MEDIATED CLAS TRANSMISSION | 2018 | 2018 | 2017-08547 | Brown, J. K. | UNIVERSITY OF ARIZONA TUCSON, ARIZONA | Brief Full |
| CITRUS UNDER PROTECTIVE SCREEN (CUPS) FOR HLB MANAGEMENT | 2018 | 2018 | 2017-08537 | Schumann, A. W. | UNIVERSITY OF FLORIDA GAINESVILLE, FLORIDA | Brief Full |
| TRANSGENIC SUCCESS-GUIDED REPRODUCTION OF HUANGLONGBING DISEASE RESISTANCE/TOLERANCE IN CITRUS BY GENE EDITING | 2018 | 2018 | 2017-08545 | Mou, Z. | UNIVERSITY OF FLORIDA GAINESVILLE, FLORIDA | Brief Full |
| DEVELOPMENT OF NON-TRANSGENIC HLB RESISTANT CITRUS VARIETIES USING CRISPR-CAS9 | 2018 | 2018 | 2017-08534 | Wang, N. | UNIVERSITY OF FLORIDA GAINESVILLE, FLORIDA | Brief Full |
| ACCELERATING IMPLEMENTATION OF HLB TOLERANT HYBRIDS AS NEW COMMERCIAL CULTIVARS FOR FRESH AND PROCESSED CITRUS | 2018 | 2018 | 2017-08555 | Baldwin, E. | ARS-USDA ATHENS, GEORGIA | Brief Full |

**ACCESSION NO:** 1014838 [Full Record]
**PROJ NO:** ARZT-XXXXXXX-G25-574 **AGENCY:** NIFA ARZT
**PROJ TYPE:** OTHER GRANTS **PROJ STATUS:** NEW
**CONTRACT/GRANT/AGREEMENT NO:** 2018-70016-27411 **PROPOSAL NO:** 2017-08547
**START:** 15 JAN 2018 **TERM:** 14 JAN 2022
**GRANT AMT:** $3,846,151 **GRANT YR:** 2018
**AWARD TOTAL:** $3,846,151
**INITIAL AWARD YEAR:** 2018

**INVESTIGATOR:** Brown, J. K.

**PERFORMING INSTITUTION:**
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***BIOPESTICIDAL DSRNA THERAPY FOR PSYLLID MORTALITY AND ABATEMENT OF VECTOR-MEDIATED CLAS TRANSMISSION***

**NON-TECHNICAL SUMMARY:** In this project, we will develop and deliver a kind of 'therapy' that relies on small dsRNA molecules that mimic psyllid gene sequences. When the dsRNAs are applied foliarly to citrus trees (phloem) and ingested by psyllids during feeding, the dsRNA initiates a response that knocks down, or silences psyllid gene expression. RNA-interference (RNAi) can result in reduced expression of genes (proteins), which in turn can impair important molecular and cellular functions in the target organism. Previously, we demonstrated that RNAi effectively silences gut gene expression in psyllids, some resulting in psyllid mortality, and others that interfere with Liberibacter invasion and establishment in the psyllid gut, resulting in reduced Liberibacter transmission. We also have shown that by using multiple dsRNAs together, or 'stacking' them, the effects can be multiplied. Stacking has the added benefit that psyllids will be unlikely to develop resistance to 'therapeutic' dsRNA treatment. DsRNA therapy offers a highly target-specific, non-toxic (humans, off-target organisms, and environmental) that does not require GMO technology for effective delivery of an effective dose to the plant, and will control CLas and ACP simultaneously, leading to reduced psyllid population size and lowered CLas levels in citrus trees. Further, highly promising light laser delivery technology has been used to create tiny wounds in the epidermis of citrus leaves, and when dsRNA is applied foliarly with effective delivery amendments (surfactants, penetrants, and protectants), the dsRNA molecules are directed into the phloem. Effective lasers are already available for laboratory and field applications, including models designed for use in citrus groves. Together RNAi and laser-enhanced foliar delivery of dsRNAs promise to provide a significant new tool to HLB management. These breakthroughs address timely needs of the citrus industry by creating new ways forward that promise to return production to near pre-HLB levels, permit re-establishment of groves in HLB hard-hit locales, aid in reclaiming HLB infected groves, and make it economical to expand new tree plantings to meet market demands. This project focuses on the design and delivery of effective, target-specific dsRNAs already proven to cause psyllid mortality or interfere with psyllid-mediated CLas transmission, following dsRNA ingestion. Herein, we couple translational research and extension pillars of discovery, design, efficacy testing, validation, and characterization, with a promising delivery system for HLB control.

**OBJECTIVES:** The goal of this project is to develop 'therapy' that uses small dsRNA molecules delivered to the plant phloem, that when ingested by psyllids, induces RNA-interference (RNAi). In a previous project, we (and others) have demonstrated that gene silencing of psyllid genes can cause high mortality, and/or interfere with Liberibacter invasion of the psyllid gut, and potentially reduce rates of transmission. The project will define optimal delivery parameters for 'best dsRNA performers' based on dsRNA dose, efficacy, and persistence criteria. The long-term goal is to implement RNAi in citrus IPM programs to reduce psyllid vector numbers and lower CLas inoculum levels. The project objectives are:Objective 1: Optimize in planta tracking of

dsRNAs delivered by laser-treated leaves and CTVvv vector inoculation. Methodologies and parameters established will inform Obj. 3-5.Objective 2: Optimize and implement tracking of dsRNA in psyllids (target) and non-target insects (whitefly) exposed to dsRNA delivered by laser-leaf treatment and the CTVvv-vector, as a crucial validation tool.Objective 3: Determine the minimal effective doses of dsRNA required to induce a measurable phenotype, e.g. mortality, transmission interference, or developmental aberration in the target psyllid species.Objective 4: Characterize the in planta persistence of the most effective dsRNAs, and with minimal effective doses in the 'fast track' PoP-CLso screen.Objective 5. Test dsRNAs with greatest efficacy and persistence, at the minimal effective doses in the ACP-citrus assay system to identify the top 2-3 dsRNAs; conduct field tests of the dsRNAs using the respective parameters.Objective 6: Conduct global large and small RNA gene expression profiling of psyllids (ACP and PoP) and whitefly (non-target) exposed to laser-foliar delivered 'top-ranking' dsRNAs, and the determine extent of dsRNA specificity.Objective 7: Develop an effective Extension program, with a focus on HLB IPM that incorporates RNAi biopesticides.

**ACCESSION NO:** 1014743 [Full Record]
**PROJ NO:** FLA-CRC-005675 **AGENCY:** NIFA FLA
**PROJ TYPE:** OTHER GRANTS **PROJ STATUS:** NEW
**CONTRACT/GRANT/AGREEMENT NO:** 2018-70016-27387 **PROPOSAL NO:** 2017-08537
**START:** 01 JAN 2018 **TERM:** 31 DEC 2021
**GRANT AMT:** $3,518,395 **GRANT YR:** 2018
**AWARD TOTAL:** $3,518,395
**INITIAL AWARD YEAR:** 2018

**INVESTIGATOR:** Schumann, A. W.

**PERFORMING INSTITUTION:**
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***CITRUS UNDER PROTECTIVE SCREEN (CUPS) FOR HLB MANAGEMENT***

**NON-TECHNICAL SUMMARY:** Citrus greening or Huanglongbing (HLB) disease makes it virtually impossible to profitably grow citrus with conventional methods. Proof of concept studies in UF/IFAS showed that high yielding trees can be grown under protective screen structures for fresh fruit production by completely excluding the Asian citrus psyllid (ACP, Diaphorina citri) and therefore HLB disease. In partnership with UF/IFAS research and extension, many Florida fresh citrus growers are rapidly adopting "Citrus Under Protective Screen" (CUPS) to ensure viable, sustainable supplies of high quality fresh citrus for Florida packing houses and consumers while keeping the U.S. industry competitive in the world

marketplace. CUPS are also being implemented in California where HLB is increasing at an alarming rate. Research/extension activities proposed in this project focus on improving CUPS by developing efficient, automated detection methods for ACP incursions, integrated pest management with biocontrol, horticultural methods such as selective canopy management, hydroponics and sensor-based irrigation, suitable varieties and rootstocks to boost yields, and a comprehensive economic analysis. Extension/outreach activities are integrated into every research activity, most notably by conducting research with growers in their own facilities. CUPS is a readily available technology that can be immediately adopted by growers to revitalize the Florida citrus industry and prevent the California citrus industry from reaching a similar stage of decline and consolidation. CUPS may reduce insecticide use and further improve premium HLB-free fruit marketability by providing consumers with lower pesticide residues than equivalent outdoor fruit, and with less impact on the environment.

**OBJECTIVES:** The proposal goal is to develop new technologies to improve sustainable fresh citrus production with CUPS and provide strong data evidence that this approach is an economically viable near-term alternative to grow HLB-free citrus. Specific objectives of this proposal are:Objective 1: Integrated pest and disease management. Hypothesis: CUPS screen selectively excludes ACP and therefore HLB disease, but allows entry of smaller pests (e.g. thrips, mites, scales), and diseases (e.g. greasy spot, citrus canker), requiring unique integrated pest management (IPM) guidelines and novel tools for management.Objective 2: Robotic machine vision with artificial intelligence (AI) to improve pest and disease scouting. Hypothesis: Camera-based scouting with artificial intelligence will allow every tree in the CUPS to be frequently examined for pests and diseases, and ensure earliest detection of ACP incursions through doorways.Objective 3: Scion and rootstock selection for CUPS. Hypothesis: High fruit yields and quality of preferred varieties for the fresh market are most profitable. Dwarfing rootstocks and self-pollinating, heat-tolerant scions are essential for high-density plantings in the pollinator-free, hotter CUPS environment.Objective 4: Horticultural improvements to maximize premium-grade fresh fruit production in CUPS. Hypothesis: The high cost of the CUPS screen house which is essential to prevent HLB, can be offset by new horticultural advances that will generate revenue with the highest quantity and quality of fruit for the fresh market.Objective 5: Evaluate the economic feasibility of fresh citrus production using different practices within CUPS under different market conditions in both Florida and California. Hypothesis: The optimal implementation of CUPS will contribute to the profitability and sustainability of fresh citrus production in HLB-affected citrus regions by excluding the ACP while increasing yields and quality of fruit.Objective 6: Develop decision support guidelines for CUPS. Hypothesis: Industry awareness and education is key for acceptance of CUPS as a solution to citrus production under HLB disease pressure. The extension and outreach program will focus on stakeholder's training and education and provide guidelines and recommendations for growing citrus undercover.

**ACCESSION NO:** 1014787 [Full Record]
**PROJ NO:** FLA-MCS-005676 **AGENCY:** NIFA FLA
**PROJ TYPE:** OTHER GRANTS **PROJ STATUS:** NEW
**CONTRACT/GRANT/AGREEMENT NO:** 2018-70016-27392 **PROPOSAL NO:** 2017-

08545
**START:** 01 FEB 2018 **TERM:** 31 JAN 2022
**GRANT AMT:** $2,951,933 **GRANT YR:** 2018
**AWARD TOTAL:** $2,951,933
**INITIAL AWARD YEAR:** 2018

**INVESTIGATOR:** Mou, Z.

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***TRANSGENIC SUCCESS-GUIDED REPRODUCTION OF HUANGLONGBING DISEASE RESISTANCE/TOLERANCE IN CITRUS BY GENE EDITING***

**NON-TECHNICAL SUMMARY:** Huanglongbing (HLB), also called citrus greening, is one of the most devastating diseases of citrus worldwide. In Florida, HLB is a disaster for the citrus industry. HLB is in Texas with spread increasing. In California, there are accelerated finds in residential trees and the insect that spreads the disease continues expanding into commercial areas. Without new solutions, it is possible that these citrus industries may follow a pattern similar to that in Florida. In Florida, HLB has spread to almost all trees, reducing yields and the quality of juice. Since 2005, when HLB was first identified, total citrus production in Florida has shrunk by more than 50%, an unprecedented contraction of the Florida citrus industry that is causing closure of citrus packing and processing plants. Citrus is the major agricultural crop in Florida, valued at $10 billion and provides 76,000 jobs. For many rural communities citrus is the economic base of the local economy. California is only second to Florida in total citrus acreage, but has a larger share of the higher value, fresh market produce. Accelerating pessimism concerning the survival of this major industry is overtaking Florida. There are no identified alternative crops to replace the use of the nearly 435,300 remaining acres of citrus and its infrastructure. Survival of the juice market in Florida requires new plantings to increase overall production. However, without resistant or tolerant citrus trees, controlling the insect that spreads the disease alone is not sufficient to provide productivity in most new plantings. California is being extremely pro-active in its efforts to prevent HLB establishment, and despite that, the insect that spreads the disease is now established. Restoration of the Florida citrus industry requires new trees that better tolerate the infection or reduce the spread of the disease and they are needed immediately. Transgenic citrus trees that are tolerant to HLB and prevent reproduction of the insect that spreads the disease have been produced. However, these trees are genetically modified organisms (GMOs) that that will have to go through the approval process that cannot be accomplished in time to save the Florida citrus industry. Additionally, the industry would have to sell a GMO product. For these reasons, we propose to recreate HLB resistance or tolerance based on the transgenic results using a new technology known as transgene-free gene editing. Citrus trees produced using this new technology will be non-GMOs and without time-consuming regulatory approval can be delivered to the industry more quickly. This will allow development of new groves that will increase the overall production levels to 2005 levels, which will increase the value of this industry and recreate jobs that have been lost in Florida and will

provide non-GMO HLB resistant or tolerant trees for the California and Texas industries that may not be ready to use transgenics. Throughout the time of this project, stakeholders will be engaged in the project development and evaluation, and the citrus communities will be informed about the transgene-free gene editing technology. Informative outreach endeavors targeting potentially interested all parties will be organized to raise awareness of the new transgene-free gene editing technology and its application in citrus and to facilitate acceptance of the resulting non-GMO citrus products. It is expected that at the end of the project, citrus growers, industry, consumers, and state/federal regulators will have understood that transgene-free gene editing technology-created non-GMO products are similar to those generated by conventional crop breeding and the market for non-GMO citrus products will be ready.

**OBJECTIVES:** The overall goal of this project is to produce citrus trees that are resistant or tolerant to HLB using the CRISPR/Cas9 gene editing technology and to educate the public (including citrus growers, consumers, and regulators) about the CRISPR/Cas9 technology and its benefits to the citrus industry. The central hypothesis of this project is that transgenic approach-generated HLB tolerance and prevention of psyllid reproduction can be reproduced by knocking out negative regulators of citrus defense signaling pathways using the CRISPR/Cas9 technology and the resulting citrus gene knockouts will be exempt from regulations. To accomplish the goal of this application, four specific objectives will be pursued(1) Identify targets for gene editing: Citrus homologous genes encoding negative regulators of a major defense positive regulator will be cloned and the citrus gene that is required for psyllid reproduction will be determined. These genes will be the priority genes and will be simultaneously subjected to CRISPR/Cas9 gene editing and CTV RNAi testing. Citrus homologs of other defense negative regulators will also be identified and tested by CTV RNAi. The most promising candidate genes will be subjected to CRISPR/Cas9 gene editing. Additionally, negative cis-element in the promoter of major defense positive regulators will be identified and tested. If promising, they will be targeted using CRISPR/Cas9.(2) Generate transgene-free citrus varieties by CRISPR/Cas9-mediated gene editing: The transformation protocols for generating citrus transgene-free CRISPR knock-outs will be optimized and transgene-free CRISPR/Cas9 gene editing on the priority genes and other promising genes identified by CTV-based RNAi will be performed.(3) Evaluate CRISPR/Cas9-produced citrus varieties: The CRISPR/Cas9 knock-outs will be tested for HLB responses in the greenhouse/field and will be molecularly characterized.(4) Engage stakeholders in project development/execution process and develop effective outreach programs: Stakeholders will be engaged in the project and outreach activities educating the public will be carried out.

**ACCESSION NO:** 1014804 [Full Record]
**PROJ NO:** FLA-CRC-005677 **AGENCY:** NIFA FLA
**PROJ TYPE:** OTHER GRANTS **PROJ STATUS:** NEW
**CONTRACT/GRANT/AGREEMENT NO:** 2018-70016-27412 **PROPOSAL NO:** 2017-08534
**START:** 15 JAN 2018 **TERM:** 14 JAN 2022
**GRANT AMT:** $3,652,166 **GRANT YR:** 2018

**AWARD TOTAL:** $3,652,166
**INITIAL AWARD YEAR:** 2018

**INVESTIGATOR:** Wang, N.

**PERFORMING INSTITUTION:**
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***DEVELOPMENT OF NON-TRANSGENIC HLB RESISTANT CITRUS VARIETIES USING CRISPR-CAS9***

**NON-TECHNICAL SUMMARY:** We propose to generate non-transgenic HLB resistant citrus varieties via genome editing of citrus susceptibility (S) genes to HLB using CRISPR/Cas9 technology. Our central hypothesis is that Candidatus Liberibacter asiaticus (Las), which utilizes a set of bacterial secreted effector proteins to manipulate key disease S gene(s), can be eliminated by modification of key S gene(s). We propose to characterize the interaction between Las effectors and key S genes or their products. Multiple candidate S genes based on interactions with key effectors have been identified in our preliminary study. We will generate mutations in the candidate S genes using CRISPR-Cas9 and screen the mutants for resistance to HLB. The confirmed S gene(s) will be modified in additional selected citrus scion and rootstock varieties. In parallel, we will construct mutations in the key S genes to pinpoint the critical residues for interactions with Las effectors, which will assist in directing plant modifications and validation of identified resistant lines. This proposal addresses one of the six areas of highest priority determined by the Citrus Disease Sub-committee (CDS): Development of tolerance or resistance to HLB in cultivars commercially important in all citrus production regions. The strategy to be employed and the objectives are developed based on input from citrus industry. Citrus grower sponsored projects established the foundation of this application, e.g., adoption of CRISPR technology on citrus. The non-transgenic plants generated here may not be regulated by USDA and could be commercialized immediately, if proven HLB resistant and with acceptable horticultural traits.

**OBJECTIVES:** In this project, we propose to characterize the interaction ofCandidatus Liberibacter asiaticus (Las)effectors and key Susceptibility (S)genes or their products. We will generate non-transgenic HLB resistant citrus varieties using CRISPR-Cas9 genome editing technology.To achieve the goals, the following hypotheses are presented and specific objectives to address these hypotheses are proposed:Objective 1. Our hypothesis is that Las requires specific secreted effectors that directly or indirectly activate key host S genes or their products, which subsequently facilitate pathogen multiplication and trigger disease symptoms of HLB. To test this hypothesis, we will conduct functional characterizations of critical virulence effectors of Las and the associated key HLB S genes using mutagenesis, yeast two hybrid assays, ectopic expression, and RNA interference techniques.Objective 2. We hypothesize that HLB resistance can be obtained by modifying key citrus HLB S genes to be unresponsive to Las. We will alter key S genes using CRISPR-Cas9/sgRNA genome editing technology to develop HLB resistant

citrus varieties.Objective 3. We hypothesize that select modified citrus varieties will have HLB resistance and acceptable horticultural traits. We will evaluate the genome-modified citrus varieties for HLB resistance, tree growth and development, canopy development, and other related horticultural traits.Objective 4. Acceptance of genetically modified citrus will be determined by the availability of educational material about the genome modification process. We propose to provide educational material and assess public sentiment to genome edited products through customer responses to educational material.Objective 5. Extension and outreach with stakeholders are hypothesized to facilitate the development and delivery of products to end-users. We propose to provide extension and educational outreach to stakeholders and consumers.

**ACCESSION NO:** 1014825 [Full Record]
**PROJ NO:** GEOW-2017-08555 **AGENCY:** NIFA GEOW
**PROJ TYPE:** OTHER GRANTS **PROJ STATUS:** NEW
**CONTRACT/GRANT/AGREEMENT NO:** 2018-70016-27453 **PROPOSAL NO:** 2017-08555
**START:** 15 JAN 2018 **TERM:** 14 JAN 2022
**GRANT AMT:** $2,922,114 **GRANT YR:** 2018
**AWARD TOTAL:** $2,922,114
**INITIAL AWARD YEAR:** 2018

**INVESTIGATOR:** Baldwin, E.

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***ACCELERATING IMPLEMENTATION OF HLB TOLERANT HYBRIDS AS NEW COMMERCIAL CULTIVARS FOR FRESH AND PROCESSED CITRUS***

**NON-TECHNICAL SUMMARY:** This research proposal aims to determine citrus scion hybrids with tolerance or resistance to citrus greening or huanglongbing (HLB) disease, that have acceptable commercial quality for fresh or processed fruit and fruit juice, respectively, and that can be classified as orange or grapefruit based on their aroma profiles. Citrus is the most economically important fruit tree crop in Florida, contributing substantially to employment, and providing cultural context for many generations of growers. Citrus had an estimated on-tree fruit value of $1.5 billion for 2006-7 prior to HLB disease, and declined to $0.82 billion for 2015-16. The value-added processed orange and grapefruit juice brings the industry value to $8-9 billion prior to this disease. Unfortunately, HLB disease came on the heels of a canker eradication effort and several hurricanes, which all together have reduced citrus production in Florida by around 70%. The disease has also been discovered in other citrus-growing states including Texas and California, and is caused by a phloem-restricted bacterium, Candidatus Liberibacter asiaticus (CLas) that is spread by the insect vector Diaphorina citri (Asian citrus pysllid or ACP). As a

result of this yield reduction, packinghouse and processing facilities are closing and at some point this infrastructure will not be enough to support the citrus industry, and will be hard to replace once gone. In addition, the disease affects the flavor quality of the fruit and resulting juice in that it reduces sugars while increasing acids, bitter limonoids and astringent flavonoids and altering the aroma profile. It is hoped that the more resistant/tolerant hybrid fruit will have improved yield and flavor characteristics. Meanwhile, to mitigate symptoms, growers are using foliar nutrition applications, pesticide sprays to control the vector and bactericides to control the pathogen, which have increased grove costs by 2.5 times or more. The project will identify cultivars and hybrids with commercially useful resistance/tolerance to HLB for near term industry use, with good flavor and other attributes, some specifically for use in juice blends that resemble orange or grapefruit juice. This should improve yield, stabilize infrastructure and reduce grove costs. Molecular markers will be identified to increase confidence that selected cultivars truly are tolerant/resistant even though they lack long term testing.

**OBJECTIVES:** The overall objective of this proposed research is to identify orange- and grapefruit-like citrus hybrids that are tolerant or resistant to HLB, as determined by molecular biology, pathology, physiology, flavor chemistry and sensory science. There appears to be no true resistance in any conventional citrus scions. Some resistance or field tolerance to HLB within citrus and citrus relatives has been described over many years prior to finding HLB in the U.S. In Florida, we have identified several released cultivars that appear to have commercially useful levels of HLB resistance and these are predominately mandarin in their pedigrees. In more distant relatives, Poncirus has been the most important source of genetic resistance, with successful mapping of genetic markers and identification of gene candidates conferring the resistance. Trees of P. trifoliata are clearly less susceptible to HLB than cultivated citrus scion varieties. P. trifoliata and its hybrid, 'Carrizo' citrange developed less severe HLB symptoms and among the lowest titer of CLas among the genotypes evaluated in a greenhouse study with a Florida isolate. To determine tolerance or resistance to HLB, molecular markers will be identified to increase confidence that selected cultivars truly are tolerant/resistant even though they lack long term testing. These markers will expedite identification of additional HLB-tolerant/resistant scions for near term use and will contribute to longer term breeding efforts. Meanwhile on the flavor side, juice from tolerant hybrids will be analysed for sugars, acids, limonoids, flavonoids and aroma volatiles for comparison to commercial orange and grapefruit cultivars. Those hybrids with commercial acceptability for fresh fruit and juice quality will be selected for grower trials. Communication with stakeholders to identify obstacles to new cultivar acceptance is planned. It is also essential to disseminate information rapidly as it arises from this proposed research. This will be accomplished efficiently via websites, presentations at industry and scientific meetings as well as through our stakeholder members of the advisory board through biannual meetings.