Hall 18-065C

High-Throughput Inoculation of Citrus Germplasm for HLB Resistance Screening

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Collaborators: Dr. Kim Bowman and Dr. Ed Stover

Duration of Requested Funding: 3 years
Total Requested Budget: $500,328

Annually:
$146,776 salaries
$20,000 supplies

Project justifications:
To inoculate new transgenic and conventional citrus developed for HLB tolerance or resistance.
Support other projects targeting ACP, Clas or vector and host relations (recently 8 lead scientists and their post docs, researching within multiple disciplines across multiple institutions)

USDA-ARS HLB Inoculation Program

Time line

1998: Psyllid found in Florida

2005: HLB discovered in Florida, already widespread

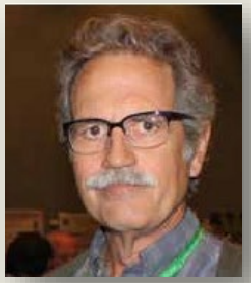
2006 – 2008: USDA citrus breeders and horticulturalists begin evaluating germplasm for HLB resistance (bud inoculations and field observations).

2008: Breeding efforts are expanded to include antimicrobial peptides (AMPs) for HLB management, using agrobacterium-mediated transformation to develop citrus germplasm expressing different AMPs.

2009 – Hall lab initiates research on HLB transmission, establishes a colony of psyllids on HLB-infected lemon plants in a walk-in chamber.

Kim Bowman
Citrus rootstocks

Ed Stover
Citrus scions



Greg McCollum
Breeding/physiology

2010 – Several hundred citrus plants developed by the breeders become old enough to begin challenging them for HLB resistance. USDA citrus breeders team up with Hall lab to inoculate the plants.

Inoculation process using infected psyllids: cage individual plants for two weeks with 20 psyllids from an infected colony.

A single technician is charged with rearing infected psyllids and inoculating plants

Kathy Moulton
Biological Science Technician

2013 – 2015: CRDF funds the inoculation program to expedite developing transgenic germplasm with HLB resistance. This grant expired summer 2015.

2015 – 2018. CRDF elects to fund a new, 3-year continuing USDA inoculation project. Two new technicians are assigned to help Kathy with inoculations.

The breeders begin sending non-transgenic material through the process. Requests for infected psyllids come in from other researchers working on HLB.

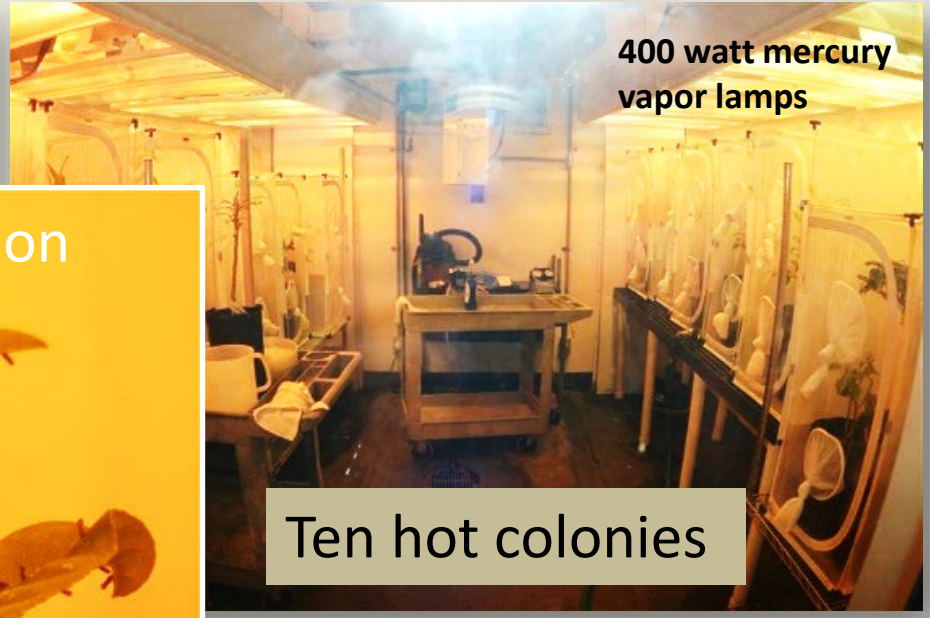
Kathy Moulton
Biological Science Technician

Ellen Cochrane
Biological Science Technician

Kerry Worton
Biological Science Technician

Establishing colonies of infected psyllids

We started out with lemon, but over time we have had 34 colonies on citron and 34 on lemon. Currently we have 25 colonies and 23 of these are on citron.



Establishing colonies of infected psyllids – walk-in chambers

Each cage with psyllids on diseased plants is a colony

Inoculating plants

Inoculation– expose each plant to 20 infected psyllids for two weeks.

Standard procedure has been to inoculate relatively young seedlings in small cone pots

Coming soon: inoculation of larger plants for two weeks with 20 or more infected psyllids

No choice detached leaf assays show no differences in resistant vs. susceptible conventional plants, but provide an excellent early screen for Liberibacter killing transgenics! This is another example of breeding projects using CLas+ ACP from this service project

Inoculations conducted 2011 – 2018:

Over 14,000 plants have gone through the inoculation process

Over 400,000 psyllids from infected colonies have been used in the no-choice inoculation step.

Labor-wise we may often be able to accommodate up to ~800 plants per month, but historically we have averaged around 350 per month.

USHRL TRANSGENIC CITRUS SCREENING PROGRAM

Psyllid Inoculation Request

PROJECT INFORMATION

Scientist Name:	
Technician Name:	Phone:
Unique name for a set of plants to be inoculated:	# plants per rep: # reps:

Plants will be kept in the Open ACP House for a period of six months. During that time the care of the plants will be provided by Dr. Hall's technicians.

Standard plant maintenance: water plants two or three times weekly; liquid fertilizer applied every three weeks; monthly applications of M-Pede; and monthly releases of predaceous mites.

In preparing for caged inoculations, minor plant trimming may be required.

Plants should normally be provided in yellow conetainers and maintained in these conetainers throughout the six month inoculation process. If for any reason plants need to be transplanted to larger pots, customer technicians will handle this.

Following a two-week caged-infestation if desired, customers can collect psyllids from these cages. However, our standard procedure will be simply to remove cages and allow the psyllids to disperse into the open

So many inoculations are run that a form was needed to keep track.

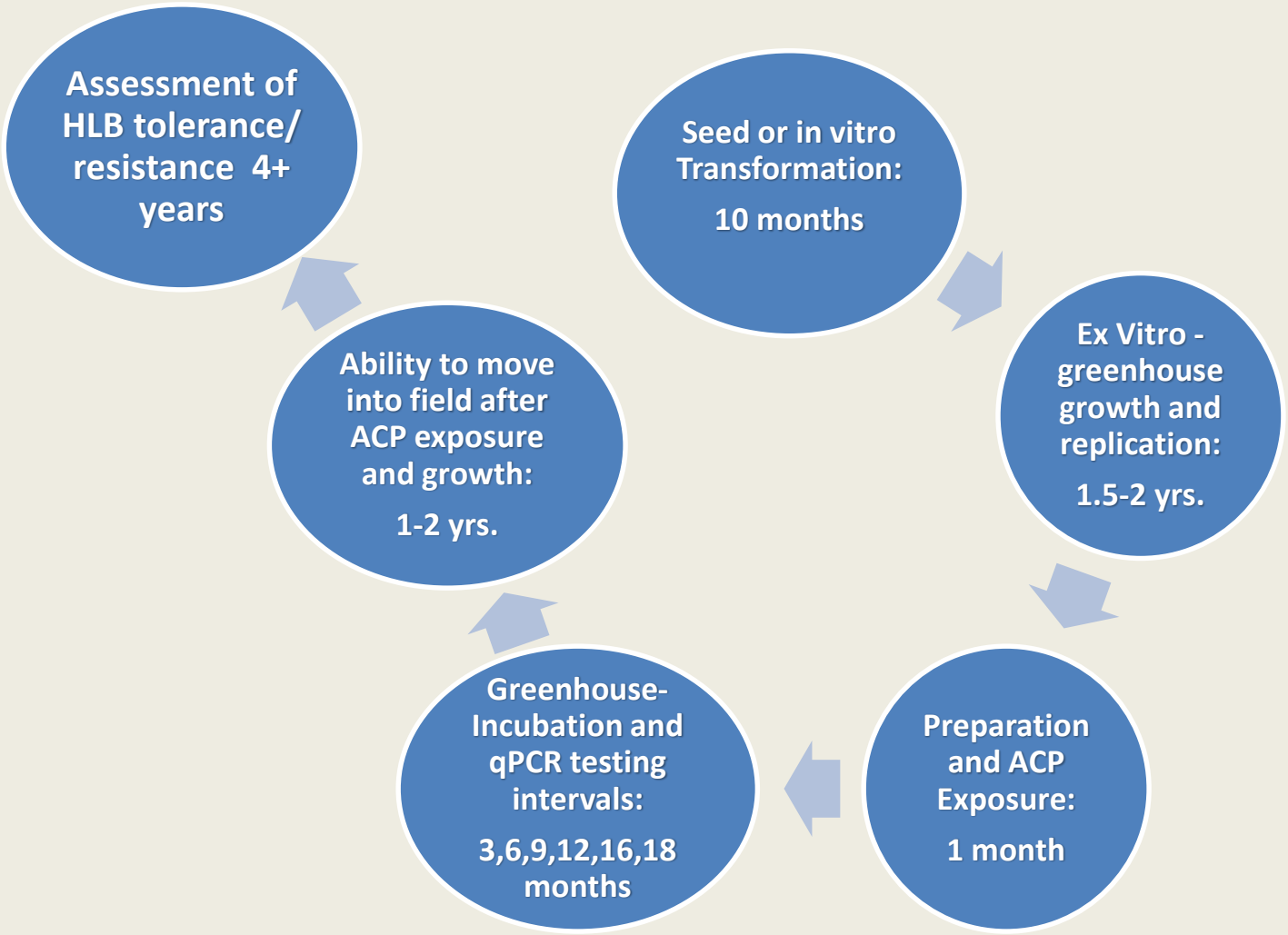
Percent ACP survival:	Last pesticide sprayed and when?
Pest problems?	

Date plants moved into the big greenhouse:

Caged Inoculations	Group 1	Group 2	Group 3	Group 4
Date plants caged and infested:				
Reps caged and infested:				
Number of ACP per plant:				
ACP Source Colony(s):				
ACP Source Plant (s):				
Psyllid survival midway through the first week (general observations)				
Date ACP removed (usually 2 weeks after ACP introduced):				
Psyllid survival at the end of the second week (general observations)				

Open ACP Infestation
Target completion date (usually 6 months after caged inoculations):

Production pipeline for transgenic (vegetative clones, in vitro) and seed-produced citrus including the ACP-facilitated Clas inoculation step.



The breeders develop groups of plants to be inoculated, usually too many to inoculate all at once. So individual replications are usually inoculated one or two at a time. Breeders keep other replications until we are ready.

Refinements to the inoculation program and evaluations of rates of inoculation have led to two scientific publications.

Journal of Economic Entomology, 109(2), 2016, 558–563
doi: 10.1093/jee/tow009
Advance Access Publication Date: 16 February 2016
Research article

Arthropods in Relation to Plant Disease

Transmission Rates of '*Ca.* Liberibacter asiaticus' by Asian Citrus Psyllid Are Enhanced by the Presence and Developmental Stage of Citrus Flush

David G. Hall,[1,2] Ute Albrecht,[1,3] and Kim D. Bowman[1]

2016 publication: the inoculation procedure works best if plants have flush.

60-95% transmission with flush, 3-40% without flush.

This contributes to our understanding of HLB epidemiology.

7% when no flush was present. In a second experiment with 'US-942', 77–97% of seedlings became infected when flush was present compared with 40% when no flush was present. A similar experiment with 'Valencia' sweet orange resulted in 23, 80, and 3% seedlings becoming infected when young, older, or no flush was present, respectively. Young plants are therefore more likely to contract HLB if flush is present, with older flush promoting higher infection rates under the conditions of this study. Based on this finding, healthy citrus should be protected from Asian citrus psyllid infestations throughout a flush. To evaluate germplasm for CLas resistance, inoculations using infected Asian citrus psyllid would best be achieved if flush is present.

Key words: *Diaphorina citri*, huanglongbing, citrus greening

Journal of Economic Entomology, XX(X), 2018, 1–7
doi: 10.1093/jee/toy240
Research

Transmission Rates of *'Candidatus* Liberibacter asiaticus' to Greenhouse Seedlings by Laboratory Colonies of Asian Citrus Psyllid (Hemiptera: Liviidae)

David G. Hall[1] and Kathryn M. Moulton

U.S. Department of Agriculture, Agricultural Research Service, U.S. Horticultural Research Laboratory, 2001 South Rock Road, Fort Pierce, FL 34945 and [1]Corresponding author, e-mail: david.hall@ars.usda.gov

2018 publication: the inoculation procedure provides a mean transmission rate of 77% (40 to 100%)

was established to expedite testing of germplasm. Colonies of Asian citrus psyllid maintained on CLas-infected plants are used for the inoculations. The primary inoculation step is to cage a healthy citrus seedling with new leaf growth for a 2-wk inoculation feeding period by 20 adult Asian citrus psyllids (no regard to sex or age) from these colonies. The adults are removed after the inoculation period, and the plants are then treated with a pesticide to eliminate any adults missed or infestation of immatures. The objective here was to evaluate inoculation rates under this procedure. Ten sets of 20 healthy citrus seedlings were subjected to the procedure, one set a month, and the percentage of seedlings successfully inoculated based on testing by quantitative polymerase chain reaction were determined 6 mo after each 2-wk inoculation period. The results indicated a 77% mean transmission rate (percent infected seedlings), with rates ranging from 40 to 100%. Transmission rates were positively correlated with the percentage of Asian citrus psyllid that tested positive for CLas but not correlated with titers of CLas in these psyllids. Possible reasons for variability in transmission rates are discussed.

Key words: *Diaphorina citri*, citrus greening, huanglongbing

Asiatic huanglongbing (HLB) is a serious disease of citrus putatively caused by the bacterium '*Candidatus* Liberibacter asiaticus' (CLas) transmitted by the Asian citrus psyllid (*Diaphorina citri* Kuwayama (Hemiptera: Liviidae)). Also known as citrus greening or yellow shoot disease, HLB can be devastating. The disease causes most commercial citrus cultivars, particularly conventional sweet oranges and grapefruit, to severely decline in health and productivity (Bové

CLas-infected Asian citrus psyllid. Some details of this program have previously been reviewed (Hall et al. 2016). Briefly, individual Asian citrus psyllid from colonies maintained on infected citrus is used to inoculate germplasm under a two-step inoculation program. The first step is a caged-infestation procedure in which individual seedlings are exposed for 2 wk to 20 Asian citrus psyllid adults from a colony reared on CLas-infected plants. The 2-wk period was

A number of challenges have been encountered!

- Ensuring plants are pesticide-free.
- Fluctuating levels of infection within colonies
- Fluctuating numbers of psyllids within colonies.

Increased requests for infected psyllids for many different research projects

For example, since May 2018:

5/25/2018	Duan	300	adults	USDA Ft Pierce	Pathology
5/25/2018	Gabriel	300	adults	UF Gainesville	Pathology
5/29/2018	Hall	3000	adults	USDA Ft Pierce	Entomology
5/30/2018	Shatters	60	adults	USDA Ft Pierce	Microbiology
5/30/2018	Stover	120	adults	USDA Ft Pierce	Miscellaneous
5/30/2018	Stover	120	nymphs	USDA Ft Pierce	Miscellaneous
5/30/2018	Stover	600	adults	USDA Ft Pierce	Breeding
6/11/2018	Stover	120	adults	USDA Ft Pierce	Miscellaneous
6/12/2018	Lapointe	20	adults	USDA Ft Pierce	Entomology
6/12/2018	Lapointe	20	nymphs	USDA Ft Pierce	Entomology
6/26/2018	Stover	600	adults	USDA Ft Pierce	Breeding
6/27/2018	Lapointe	30	adults	USDA Ft Pierce	Entomology
6/27/2018	Shatters	120	adults	USDA Ft Pierce	Microbiology
6/27/2018	Shatters	50	nymphs	USDA Ft Pierce	Microbiology
6/27/2018	Stover	600	adults	USDA Ft Pierce	Breeding
6/27/2018	Stover	120	adults	USDA Ft Pierce	Miscellaneous
6/27/2018	Stover	600	adults	USDA Ft Pierce	Breeding
6/27/2018	Stover	120	adults	USDA Ft Pierce	Miscellaneous
7/5/2018	Stover	120	adults	USDA Ft Pierce	Miscellaneous
7/9/2018	Stover	120	adults	USDA Ft Pierce	Miscellaneous
7/11/2018	Stover	120	adults	USDA Ft Pierce	Miscellaneous
7/19/2018	Stover	120	adults	USDA Ft Pierce	Miscellaneous
7/24/2018	Shatters	300	adults	USDA Ft Pierce	Microbiology
7/24/2018	Stover	120	adults	USDA Ft Pierce	Miscellaneous
7/25/2018	Stover	600	adults	USDA Ft Pierce	Breeding
7/26/2018	Heck	200	adults	USDA Ithaca	Genetics
7/26/2018	Shatters	200	adults	USDA Ft Pierce	Microbiology
7/26/2018	Stover	600	adults	USDA Ft Pierce	Miscellaneous
7/30/2018	Chandrika	50	adults	University CA	Genetics
7/30/2018	Stover	120	adults	USDA Ft Pierce	Miscellaneous
8/1/2018	Lapointe	30	adults	USDA Ft Pierce	Entomology

Thank you!

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Photo by National Geographic