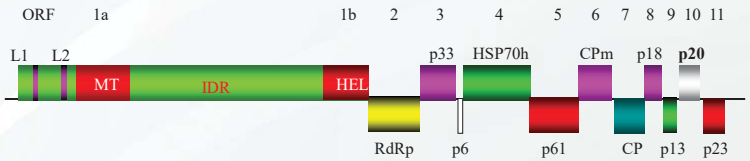
Use of CTVvv-RNAi to control Asian Citrus Psyllid

Overview

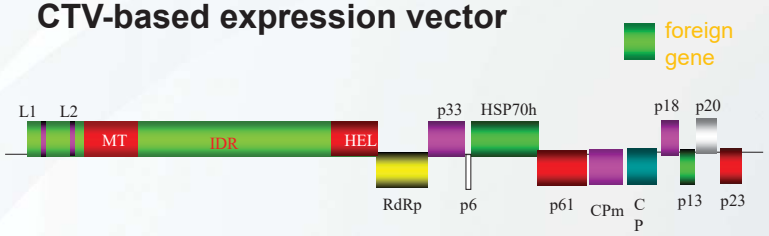
- Goal = proof of concept; validate the use of CTVvv for delivery of RNAi in the field
  - Already achieved in greenhouse
- CTVvv-RNAi as biocontrol
  - Flexible
  - Quicker to produce than transgenics
  - Field deployable
- Initial results have shown activity with multiple candidate sequences
  - Reduction in ACP multiplication
  - Possible reduction in acquisition of *CLas*

CTV vector construction

CTV genome



CTV-based expression vector



Objectives

1. Determine the efficacy of selected RNAi constructs mediated by CTVvv in controlling ACP on field grown trees
2. Determine the effectiveness of CTVvv as a delivery method of RNAi throughout the year
3. Determine the effect of CTVvv-RNAi on the acquisition and transmission of *CLas* by ACP
4. Determine the effect of CTVvv-RNAi on the spread of HLB

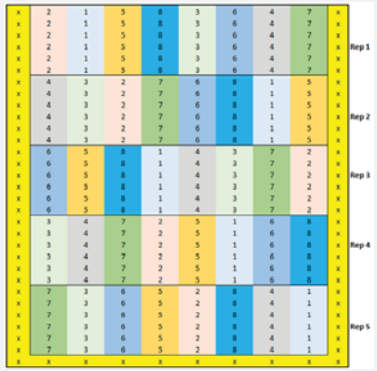
Experimental design

- Small scale trial
  - 7 sequences + control= 8 treatments X 6 tree plots X 5 replications = 240 trees (not including sentinel trees)
    - RNAi expression to be measured with additional trees carrying phytoene desaturase and GFP construc
  - Young trees inoculated prior to release (blind buds, infection status verified prior to release)
  - Minimal insecticide applications
  - Data collection:

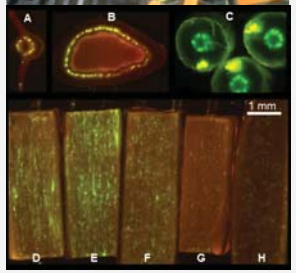
Years 1-2
- Caged infected ACP (3 trees/plot), measure *CLas* acquisition
- Natural ACP colonization (3 trees/plot)

Years 2-3
- As trees become HLB infected: caged ACP to measure *CLas* acquisition
- % HLB infection

Plot diagram

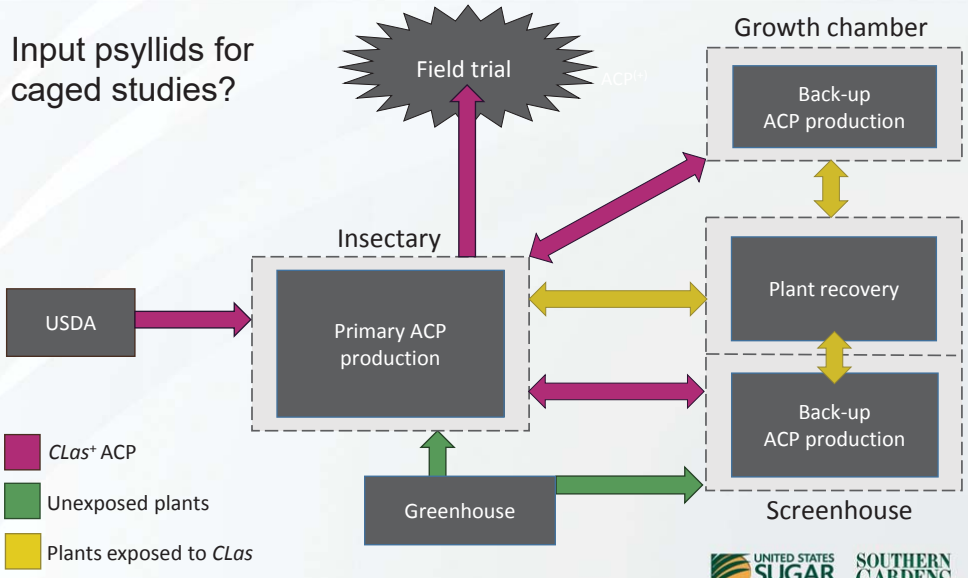


RNAi expression reporters:
- phytoene desaturase
- green fluorescent protein (GFP)



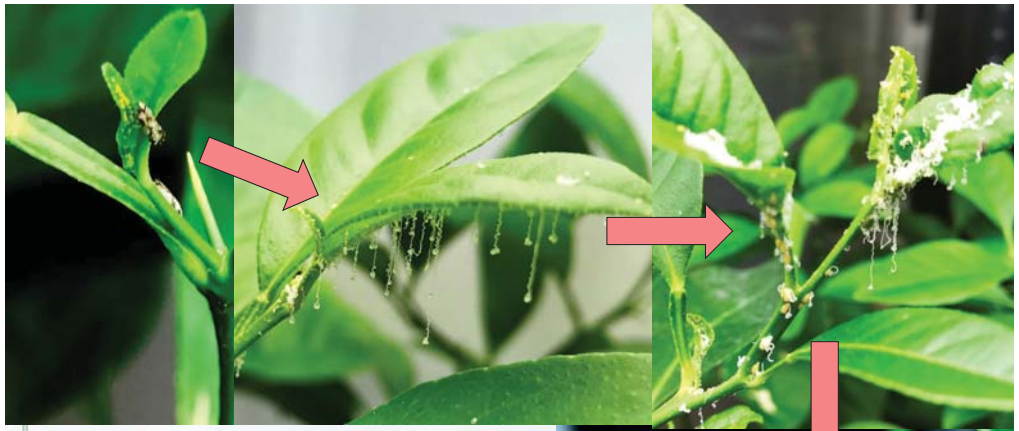
ACP mass-rearing

Input psyllids for caged studies?



Insectary protocol

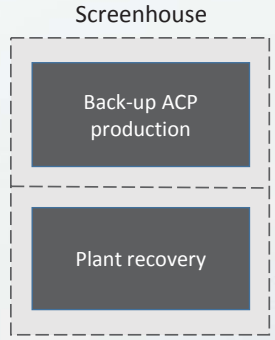
1. Obtain *CLas*$^{(+)}$ ACP from USDA colony
2. Establish supply of healthy plant material in greenhouse
   - Rough lemon seedlings
   - Tolerant to HLB; high flush production
3. Establish insectary colony
   - Introduce ~100 ACP to 10 plants per cage



4. Remove F_0 adults after 5 to 7 days
5. Collect F_1 adults after 14 to 28 days
6. Introduce same-age F_1 adults to sleeved trees in field trial

Supplementary facilities

- Divided screenhouse
  1. Psyllid house = back-up colony
     - Open infestation
     - Supply of *CLas*-exposed plants
  2. Plant recovery area
     - Supply of ACP-free, *CLas*-exposed plants
- Growth chamber = back-up colony
- ACP colonies and *CLas*-exposed plants routinely tested for % HLB infection



Field trial details

- 3 trees per plot will be caged with ACP (3-4 times/yr)
  - Determine effect(s) on ACP reproduction and survivorship

Modified Bioquip cage

Caged-tree survey:

- Cages placed over flush and 25 input ACP introduced
- After 15 days, record surviving ACP and remove
- After 30 days, record adult progeny produced and % HLB positive
- Sample of ACP to be used in field trial will be tested for % HLB infection prior to introduction to cage

- 3 trees per plot will be left uncaged
  - Uncaged tree survey (monthly):
    - Determine effect(s) on natural ACP colonization
    - Record presence of ACP developmental stages
    - Record incidences of effect(s) on non-target species
    - Record presence of other citrus pests
- Additional data
  - Monitor stability of CTVvv-RNAi constructs seasonally
  - Monitor % HLB infection of trial trees biannually
  - Monitor spread of CTVvv in sentinel trees biannually

Data summary

Data element	Sample	Number of times per year	Type of test
% HLB infection	ACP mass rearing colony	3-4	qPCR
	Trial ACP	3-4	qPCR
	Trial tree	2	qPCR
CTV titer	Trial tree	2	ELISA
	Sentinel tree	2	ELISA
Stability of RNAi inserts	Trial tree	3-4	RTPCR/gel electrophoresis
% mortality	Trial ACP	3-4	Survival counts of initial ACP and progeny
Rate of ACP reproduction	Trial ACP	3-4	Survey for proportion of ACP developmental stages
Rate of natural ACP colonization and non-target effects	Uncaged trial tree	monthly	Survey for proportion of ACP developmental stages; survey of non-targets
Presence of other citrus pests and diseases	All trees	monthly	Survey for additional citrus pests (brown aphid, citrus leafminer, etc.)

RNAi – Who owns it

- CSIRO – Commonwealth Scientific and Research Organization
  - Australian Government Corporation
    - BOD and CEO
  - 1800 Patents
    - 4 patent families around RNAi
    - Basically if you are going to commercialize/research RNAi you will need FTO around CSIRO patents.
- SCG and CSIRO
  - CTV
  - GE
  - Foliar applications