Non-transgenic*, near-term RNA interference-based application strategies for managing *Diaphorina citri* and citrus greening/Huanglongbing (HLB)

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[1] University of California, Davis; [2]University of Florida; [3]University of California, Riverside; [4]University of California, Berkeley; [5]USDA ARS, Fort Pierce, FL

***Transgenic approaches are excellent in the long run, but with HLB we don't have time.**

How will we do this?

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With plant and insect viruses!

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RNAi results in mRNA degradation.

We will use viruses to induce RNAi against the Asian citrus psyllid, *Diaphorina citri* and induce negative phenotypes, thereby helping to manage HLB spread.

Objectives

Objective 1). Optimize the Florida CTV to deliver efficacious* D. citri*-interfering RNAs in Florida non-transgenic citrus. (UFL and ARS, FL)

Objective 2). Develop a CTV vector for applications in California citrus. (UC Riverside)

Objective 3). Develop* D. citri*-infecting viruses for non-plant-based induction of RNAi effects in psyllids. (UC Davis)

Objective 4). Model/test RNAi systems under greenhouse and/or field conditions. (UFL, Gainesville)

Objective 5). Evaluate the economic impact of using RNAi technologies in citrus for controlling* D. citri *and HLB. (UC Davis)

Objective 6). Develop effective Extension Outreach programs for RNAi-based strategies. (UCR, UCB and UFL)

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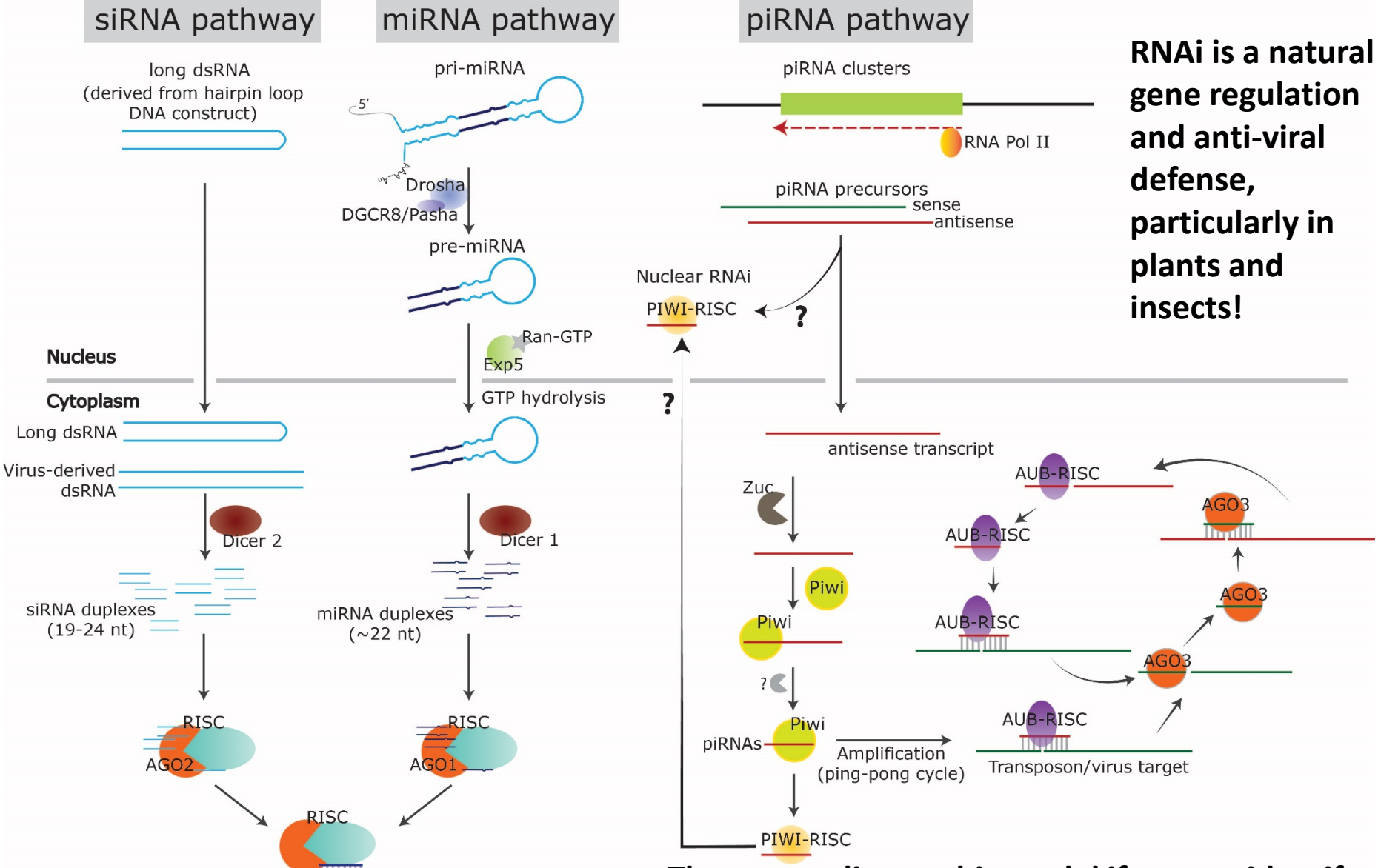


RNAi is a natural gene regulation and anti-viral defense, particularly in plants and insects!

Thus, according to this model if we can identify correct targets and then supply an effective interfering RNA (dsRNA or siRNA), we might be able to induce specific and effective RNAi effects in the target organism, *D. citri.*

Papaya ringspot virus in Hawaii

The solid block of green papaya trees are 'UH-Rainbow' while the surrounding papaya trees that are nearly dead are non-transgenic papaya trees severely infected by PRSV

The progress of the disease caused by PRSV in rows of non-transgenic papaya (left in picture) as compared to the resistance in rows of 'UH Rainbow' (right in picture).

RNAi is currently used in commercial U.S. agriculture.

But RNAi is not limited to plants, or only for plant anti-viral defense.

RNAi is a normal gene regulation and antiviral defense found in many different types of organisms including plants, animals, fungi, invertebrates, etc.

It can be induced by double-stranded (ds) or small (s) RNAs of known sequence and yield degradation or translational interference of target (m)RNAs.

RNA interference – now one of the most widely studied areas in biology with potential practical applications from medicine to agriculture.

RNAi for insect-proof plants. *Nat. Biotechnol. 2007 25: 1231 – 1232.*

Science 16 August 2013:
Vol. 341 no. 6147 pp. 732-733
DOI:
10.1126/science.341.6147.732
•News

A Lethal Dose of RNA

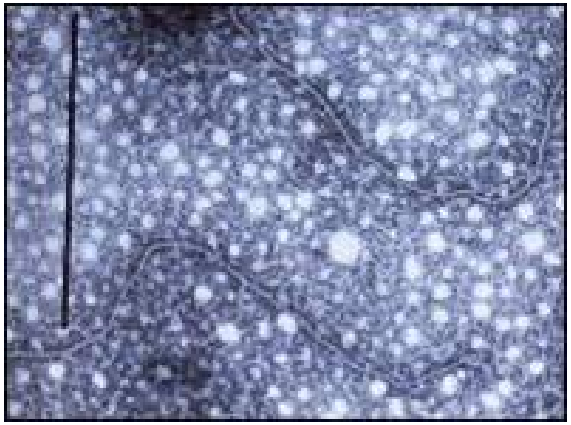
1.Kai Kupferschmidt

A new generation of genetically modified crops will kill insects by silencing their genes.

When Andrew Fire and Craig Mello won a Nobel Prize in 2006 for a revolutionary technique to silence genes, there were high hopes that the discovery would lead to new treatments for disease. RNA interference (RNAi) might help tackle a wide variety of ailments, such as virus infections, cancer, and cardiovascular disease, the Nobel committee noted. Seven years on, the technology is almost ready to be applied—but rather than healing humans, it will kill insects.

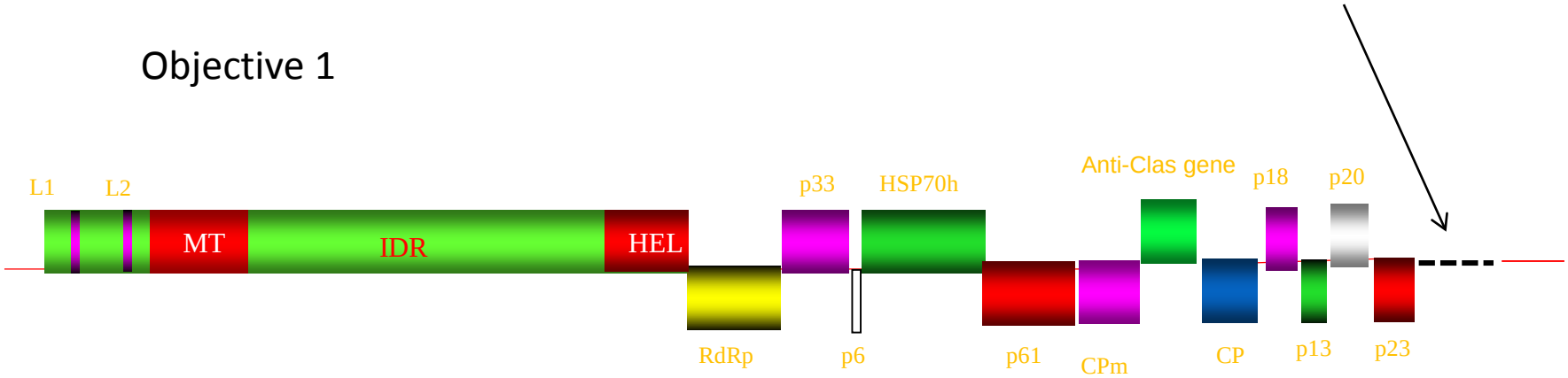
Citrus tristeza virus, a phloem limited virus that is limited to citrus and can be manipulated.

William Dawson, Nabil Killiny, Robert Shatters
University of Florida, Lake Alfred and USDA ARS Fort Pierce, FL.



Target sequence for psyllid gene inserted into CTV

Objective 1



Thus, here we use a CTV isolate that itself does not induce disease symptoms, and is very poorly aphid transmissible.

Theoretical virus-based delivery of siRNAs to phloem

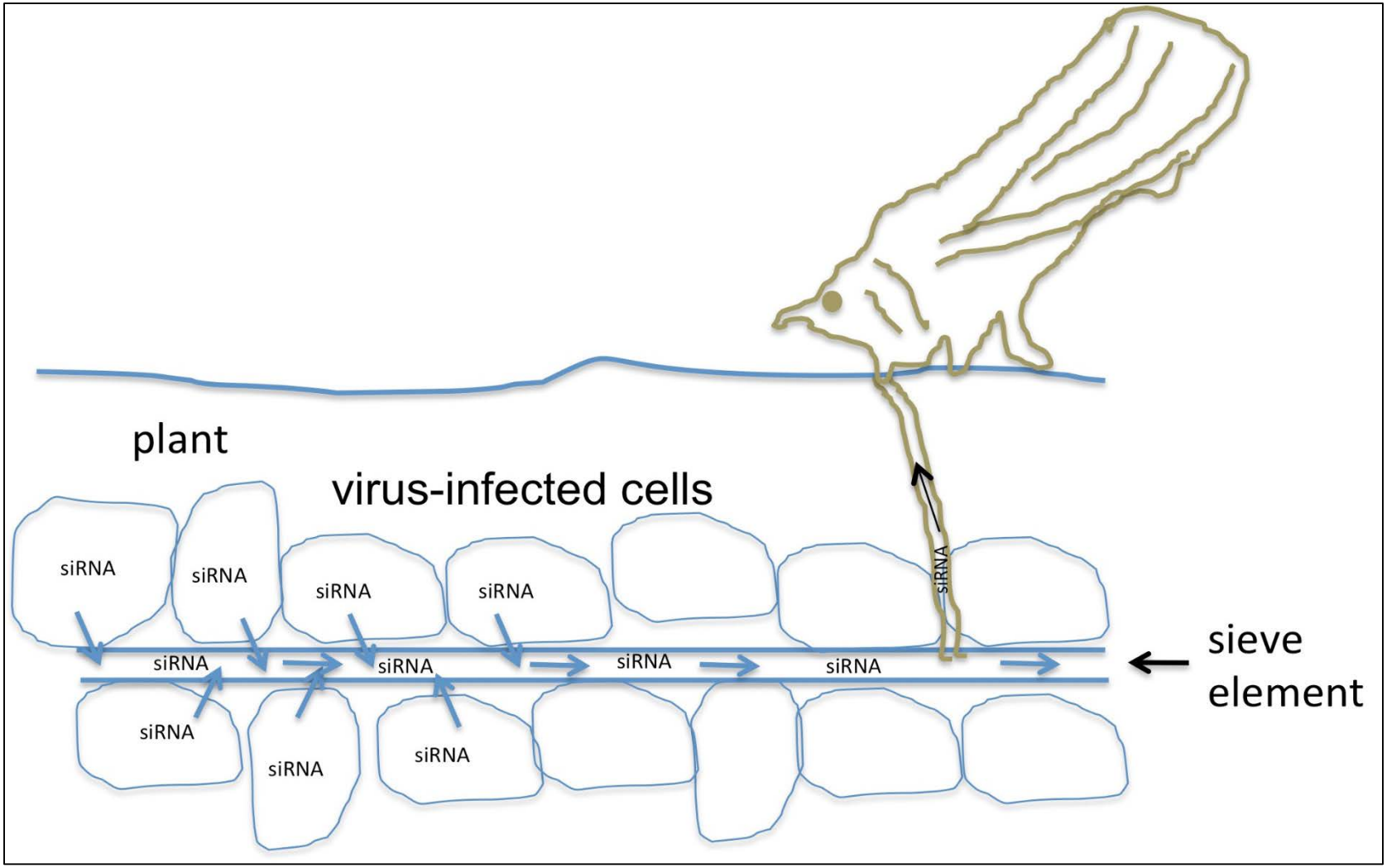
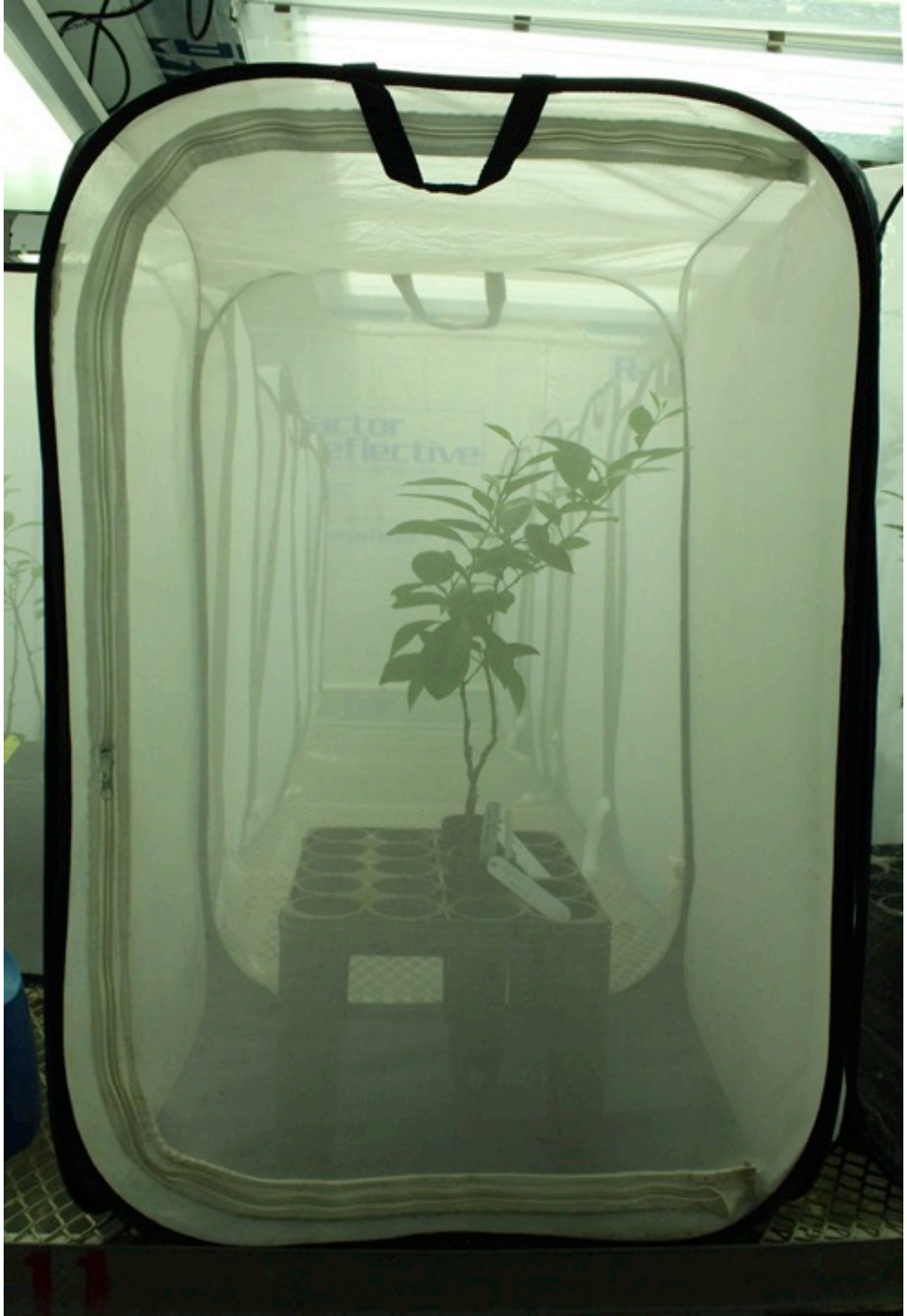


Figure 1. Cartoon of psyllid feeding and acquiring siRNAs.

Courtesy of Dr. W. O. Dawson



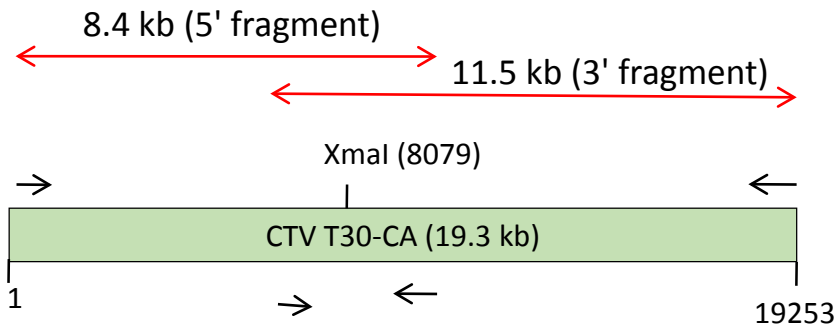
SUMMARY OF RNAi CAGE EXPERIMENTS – PSYLLID PROGENY

Construct	# of reps	Input % PCR+	Output % PCR+	Output number
control	19	24	68	300-400
ATPase	5	45	0	15
DF	9	43	2	66
HSP70	3	27	0	17
Rieske	5	44	22	32
JHE	5	33	7	6
BS-4	2	33	0	0
Osiris	5	52	0	24
JAMAT	2	---	0	6
PC	2	50	0	5

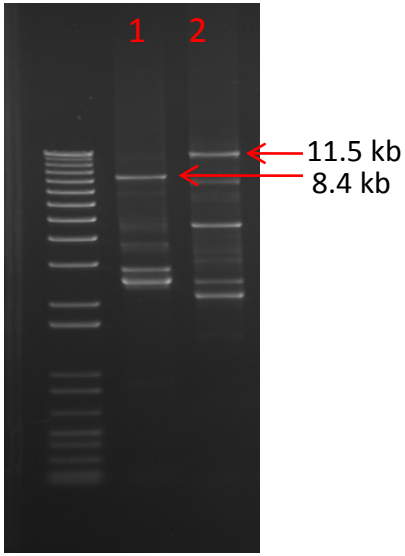
This is using the Florida CTV, we are also engineering a California CTV.

Generating the cDNAs that correspond to two overlapping regions of the CTV (T30-CA and T36-CA) genome – work done by Ng Lab, UC Riverside Objective 2

A) Amplification of CTV T30-CA 5' and 3' fragments

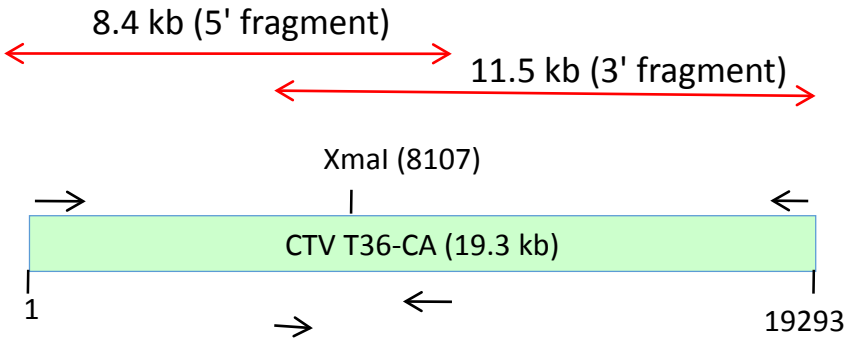


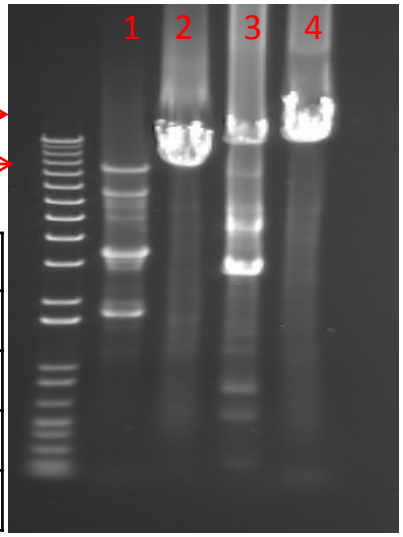
= Oligonucleotide primers



Lane	Template	Size (kb)
1	dsRNA of T30-CA	8.4
2	dsRNA of T30-CA	11.5

B) Amplification of CTV T36-CA 5' and 3' fragments



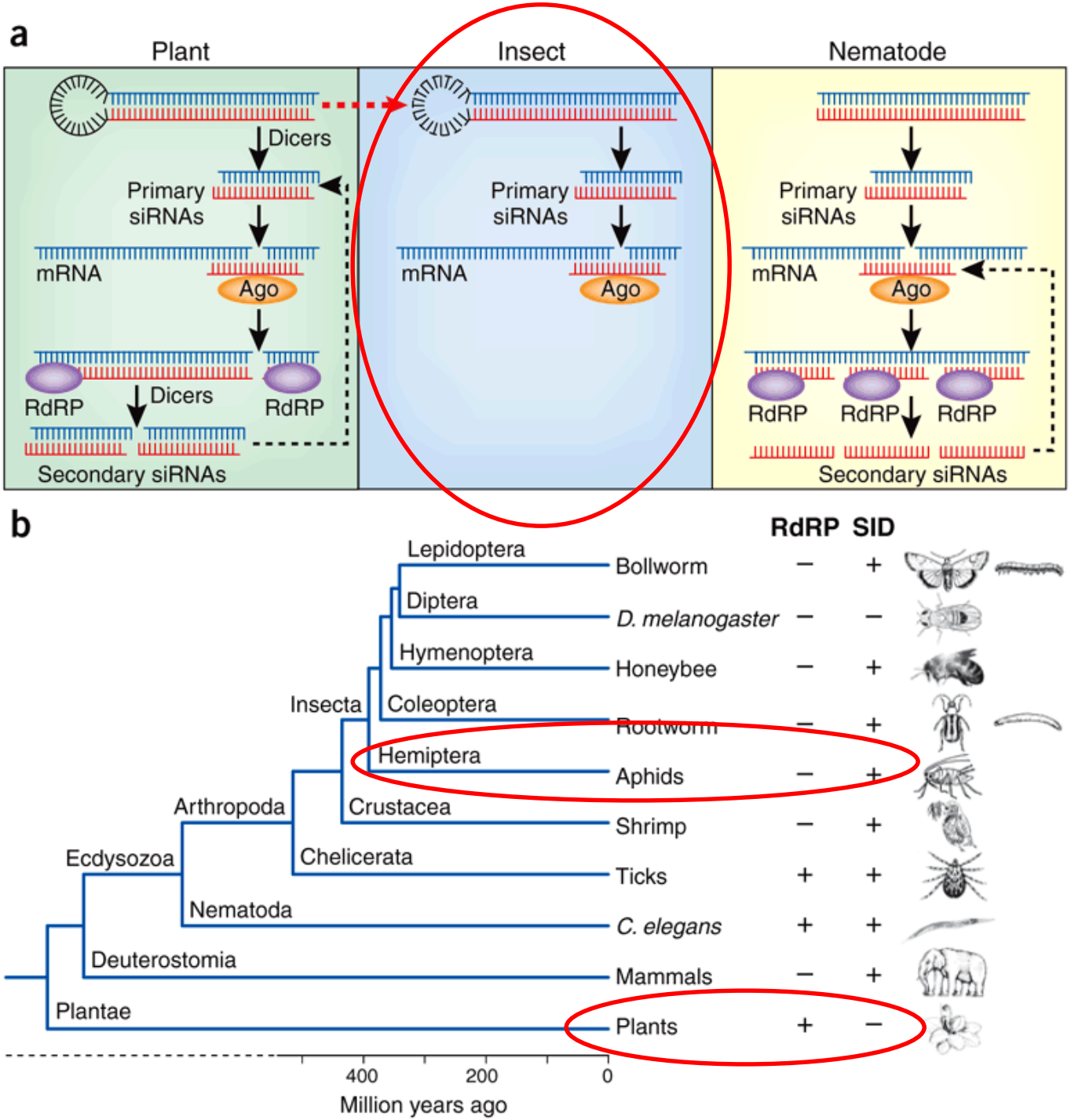


Lane	Template	Size (kb)
1	dsRNA of T36	8.4
2	pCAMCTV947R*	8.4
3	dsRNA of T36	11.5
4	pCAMCTV947R*	11.5

* the infectious clone of T36 from Florida

But RNAi is different in plants and insects.

In insects, it may not spread through the body.



http://www.acgov.org/cda/awm/agprograms/pestexclusion/sharpshooter.htm

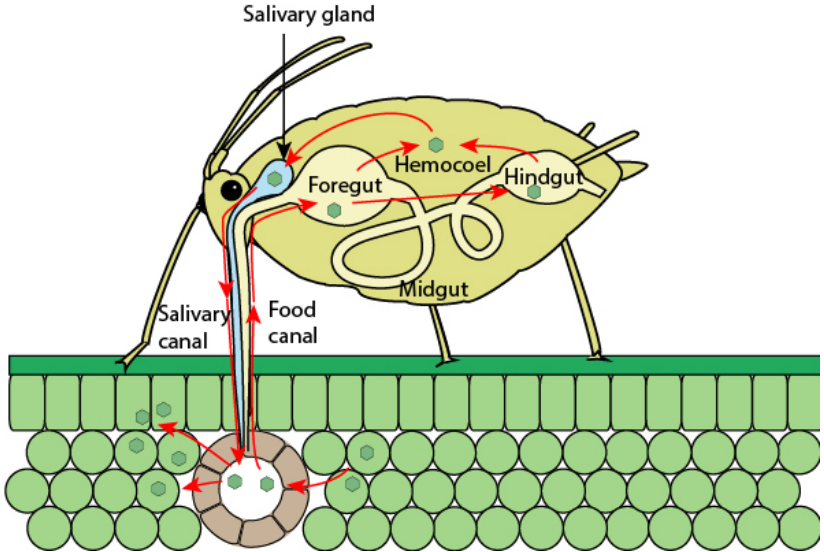
RNAi for insect-proof plants.
Nat Biotechnol. 2007 Nov;25(11):1231-2.

But insect-infecting viruses do spread systemically throughout the insect body.

Insects respond to virus infections by RNAi responses.

But only one virus so far has been reported from the Asian citrus psyllid, *D. citri.*

But it is likely that there are many viruses of *D. citri*, and perhaps one or more could be used to induce systemic RNAi responses in *D. citri.*



http://viralzone.expasy.org/all_by_protein/3738.html

D. citri RNA samples analyzed by NGS and RT-PCR for new viruses

Table 2. *D. citri* RNA samples analyzed by NGS and RT-PCR for new viruses.

Sample	Collector	NGS Platform		Identified Virus*		
		HiSeq small RNA	MiSeq RNA-seq	DcIV	DcRV	DcDV
Taiwan 1	H.H.Y.	+	-	+	+	+
Taiwan 2	H.H.Y.	-	-	-	+	+
China 1	YC HW	+	+	+	+	+
China 2	YC HW	-	-	+	+	+
China 3	YC HW	-	-	+	+	+
China 4	YC	-	-	+	+	ND**
China 5	YC	-	-	+	-	ND
Brazil 1	TS	+	-	+	-	+
Brazil 2	DMG	-	-	+	-	+
Pakistan	AMK	-	-	-	-	+
Florida 1	WOD	+	+	-	+	+
Florida 2	WOD	-	-	-	+	+
Florida 3,4,5,6	KPS	-	-	+/-	+	ND
Florida 7,8,9,10,11, 12	KPS, BF, RB	-	-	-	+	ND
Puerto Rico 1, 2, 3		-	-	-	+	ND
California 1	DM	-	-	-	-	+
California 2	DM	-	-	-	-	+
California 3	DM	-	-	-	-	+
California 4	DM	-	-	-	-	+
Hawaii-Aiea	CH	-	-	-	+	+
Texas 1	AC	-	-	-	-	+
Texas 2	AC	-	-	-	-	+
CRF	KG	-	-	-	-	+

*Viruses confirmed by RT-PCR and Sanger sequencing.
** Not done.

Falk lab, UC Davis

Viruses confirmed by RT-PCR and Sanger sequencing.

New Florida and Puerto Rico samples arrived just last and this week.

James Keesling, Jo Ann Lee, Ross Ptacek
University of Florida, Gainesville

Model Summary, Objective 4

- We model the dynamics of the psyllid and flush life cycles in a block of trees
- C.Las enters the tree through the flush as discovered by Dawson and reported in Lee et al. (2015)
- The key piece to be developed is the relationship between abundance of C.Las and development of HLB symptoms

Symptom Development

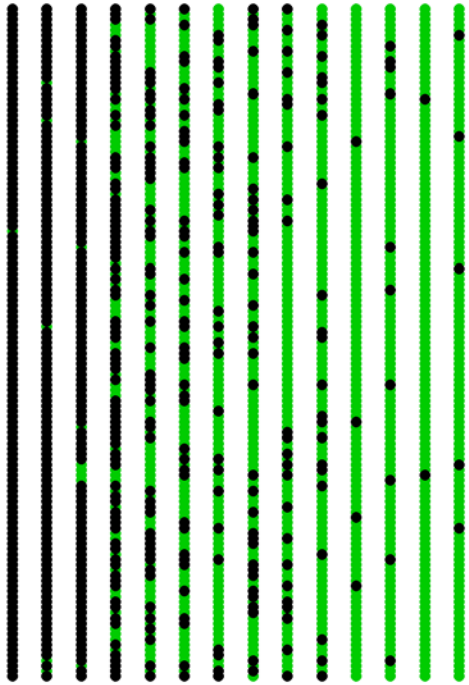
- The model tracks transmission of inoculum from psyllid to tree.
- We Hypothesize that the rate of symptom development is proportional to the presence of inoculum in the tree.
- Using data from Southern gardens we have made a first approximation for the parameters of this model.

Simulation Summary: Status of the block at the end of 10 years under various ACP control strategies

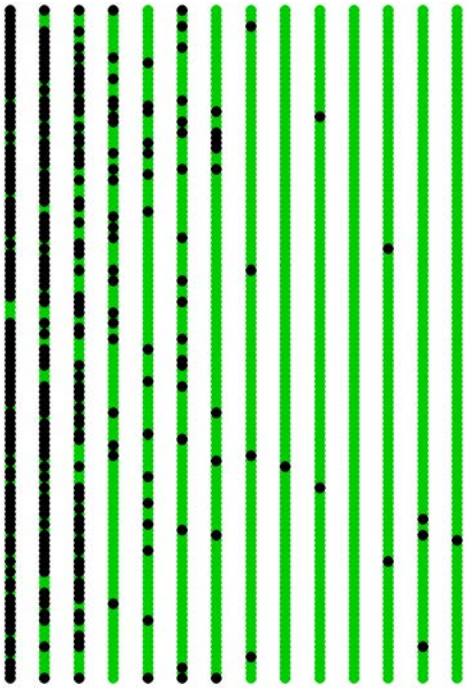
Healthy

Declining

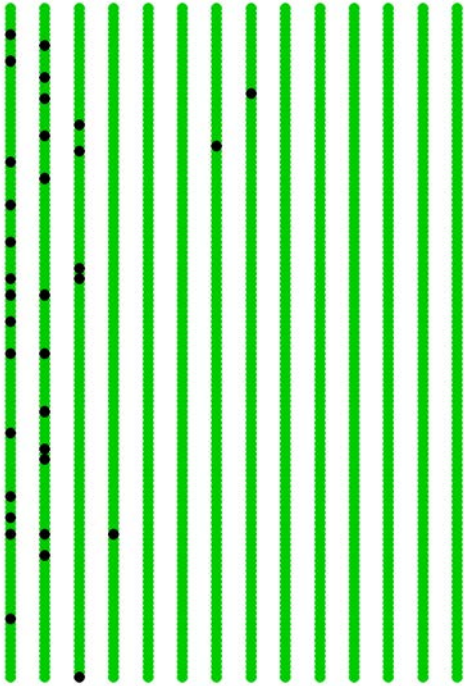
ACP Control: Spray every 4 weeks



ACP Control: RNAi



ACP Control: Spray every 4 weeks and RNAi



RNAi = 2/3 reduction of adults over a 15 day period and 80% reduction of nymphs that would normally survive to adulthood

Economic Approach

Karen Jetter, UC Davis

Objective 5

- Literature review was completed on the different approaches that have been used to incorporate economics into invasive species analyses.
- 3 approaches have been used in general.

  - 1. Optimal Control Models
  - 2. Simulation models.
  - 3. Two period equilibrium displacement models.

Economic Approach

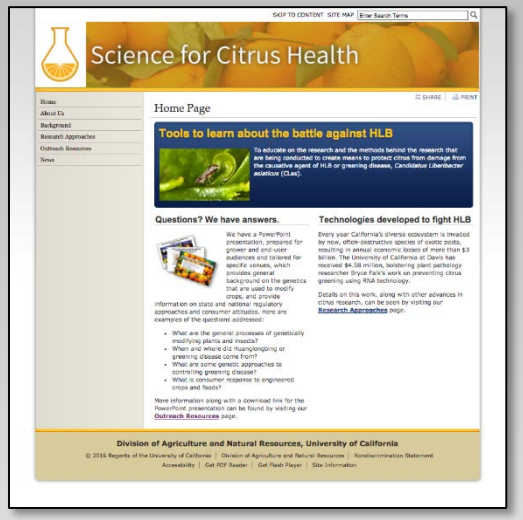
- Each approach will require baseline information on current production and costs of inputs and outputs for Florida and California.

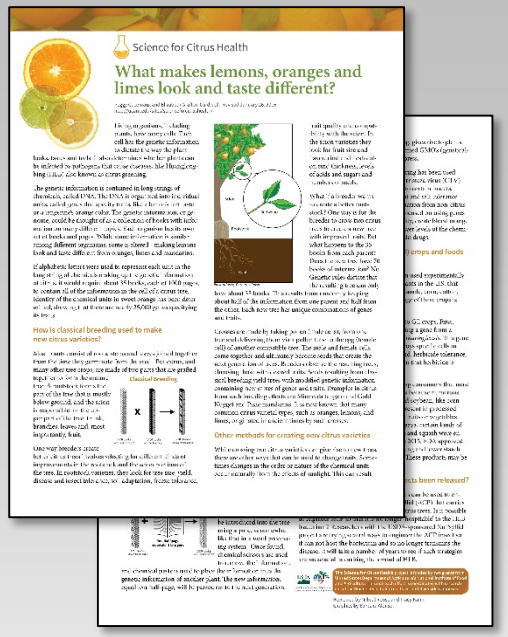
- Completed data collection on averages for 2011-2013 based on the data availability for each state and in each production region within each state.

Science for Citrus Health

Lemaux, UC Berkeley and Grafton-Cardwell, UC Riverside

Objective 6





The outreach group is working on a PowerPoint, web site and handouts that explain what genetic engineering is, using examples from citrus plant, insect and disease systems. For protecting citrus against pests and diseases, we are describing both GE and non-GE tactics.