Asian Citrus Psyllid (ACP) Research and Management

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Psyllid infestation reduces tree health with and without HLB—the combo is lethal

HLB +
No ACP

HLB +
Pulsed (monthly) ACP

HLB +
Continuous ACP

HLB –
No ACP

HLB –
Pulsed (monthly) ACP

HLB –
Continuous ACP

As a ‘stop-gap’, ACP management relied on intense insecticide use

Typical calendar of applications 2010-18

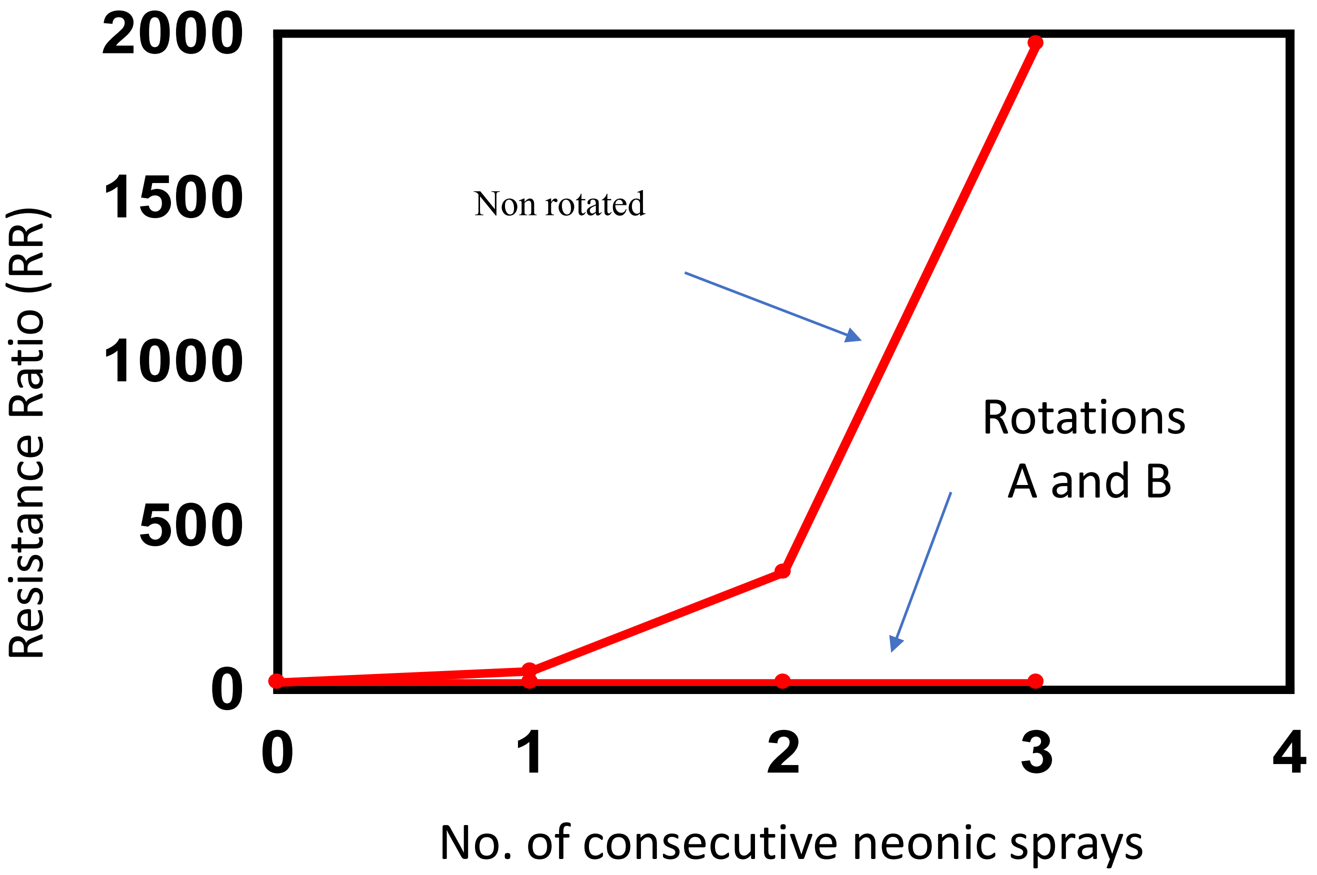
Dates	Insecticide	Aim	Cost per acre ($)
January 19, 2012	Zeta-cypermethrin (Mustang)	ACP control	**28.7**
March 13, 2012	Spirotetramat (Movento MPC)	ACP control	**62.6**
March 13, 2012	Chlorpyrifos (Lorsban 4EC)	Overspray	**48.0**
April16, 2012	Diflubenzuron (Micromite 80WGS)	ACP control	**62.3**
May 24, 2012	Spinetoram (Delegate WG)	ACP control	**61.8**
June 22, 2012	Abamectine (Agri-Mek SC)	ACP control	**39.2**
August 3, 2012	Imidacloprid (Admire Pro)	ACP control	**55.4**
August 30, 2012	Dimethoate (Dimethoate 4E)	ACP control	**29.6**
October 12, 2012	Fenpyroximate (Portal)	ACP control	**39.3**
December 14, 2012	Zeta-cypermethrin (Mustang)	ACP control	**28.7**

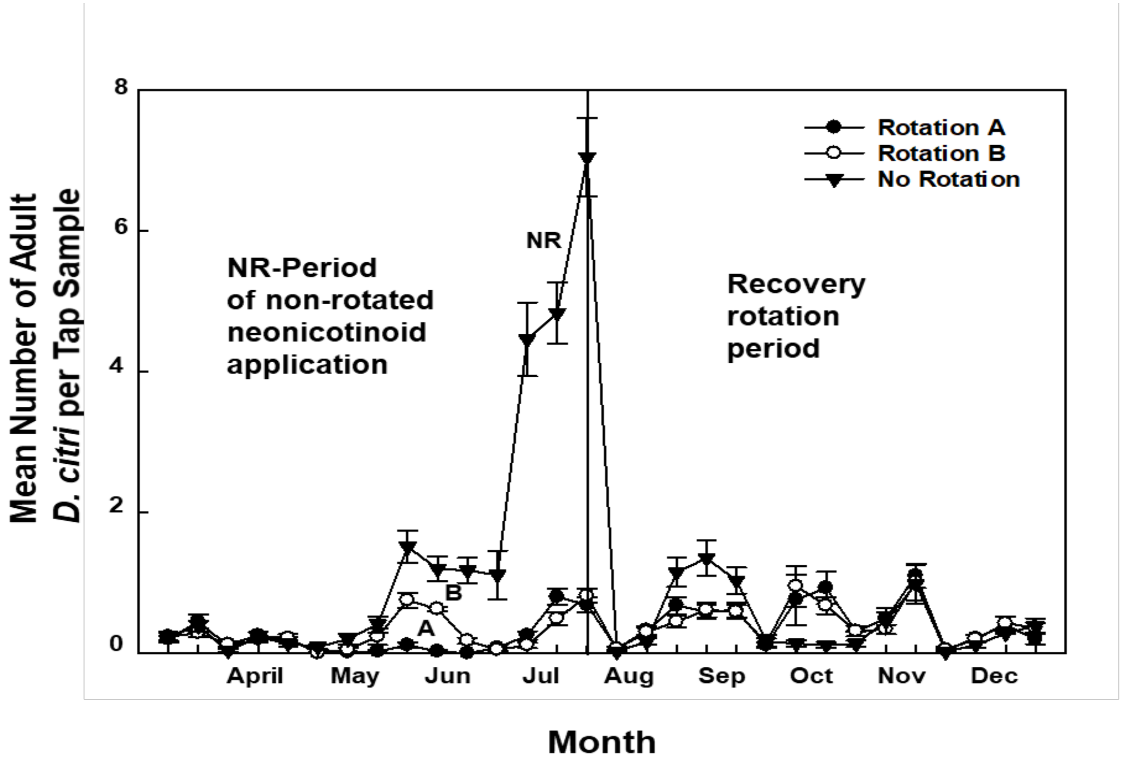
- **Expensive**
- **Eliminated populations of natural enemies**

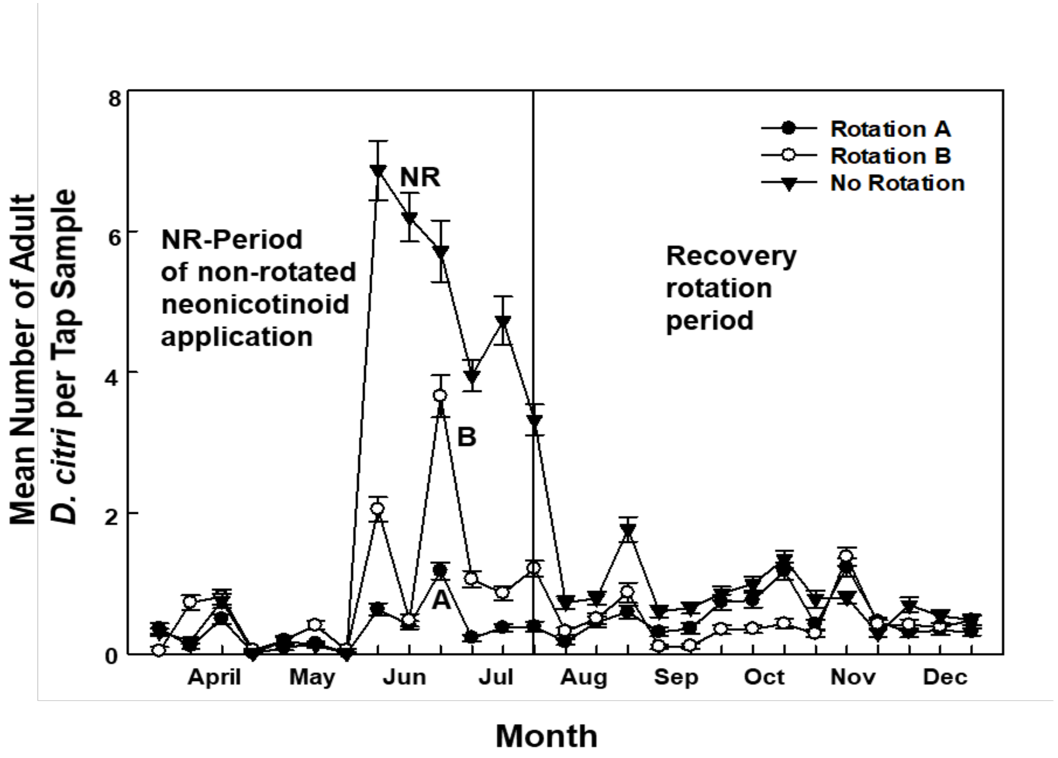
- ~2000-fold increase after 3 consecutive treatments

- ***Failure to control ACP adults is coincident with development of neonicotinoid resistance***

Resistance to thiamethoxam:

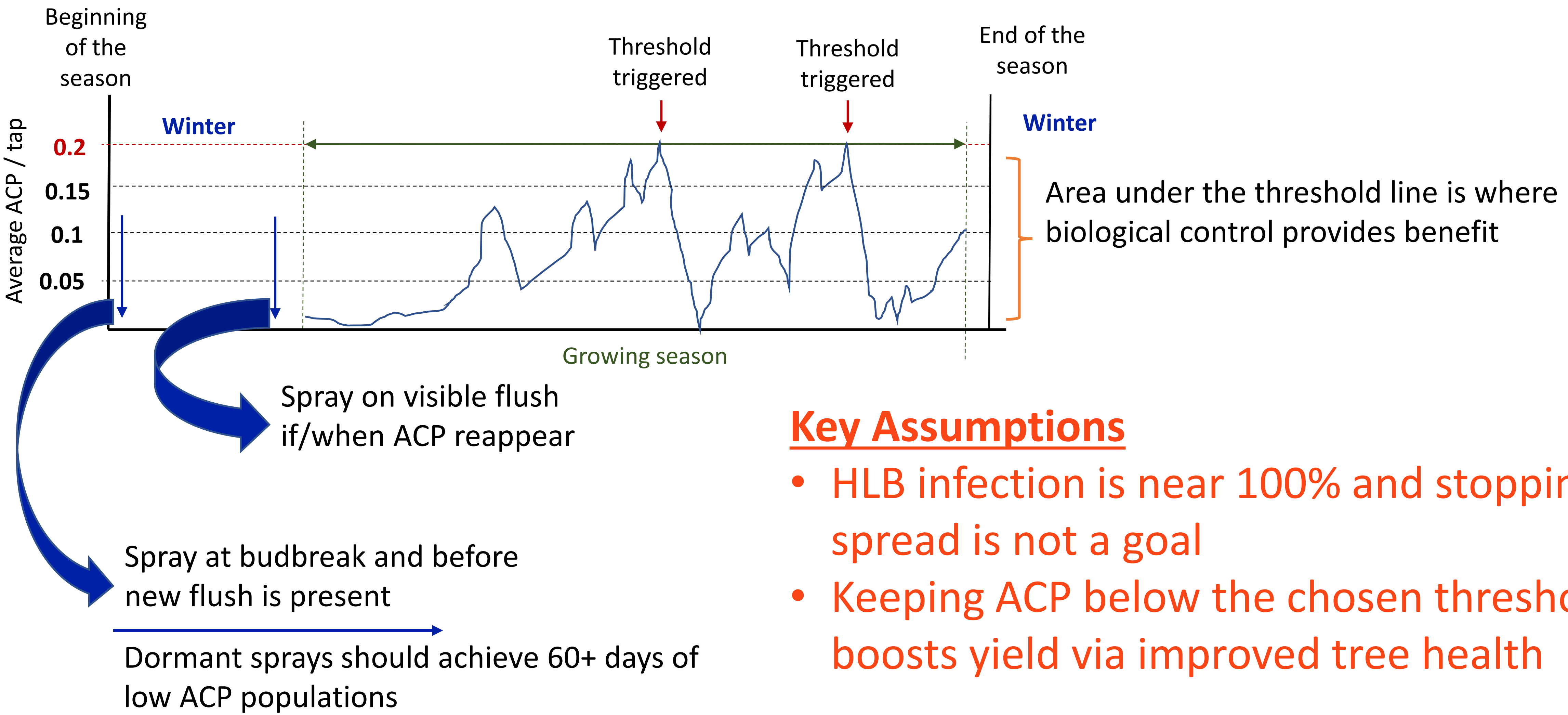






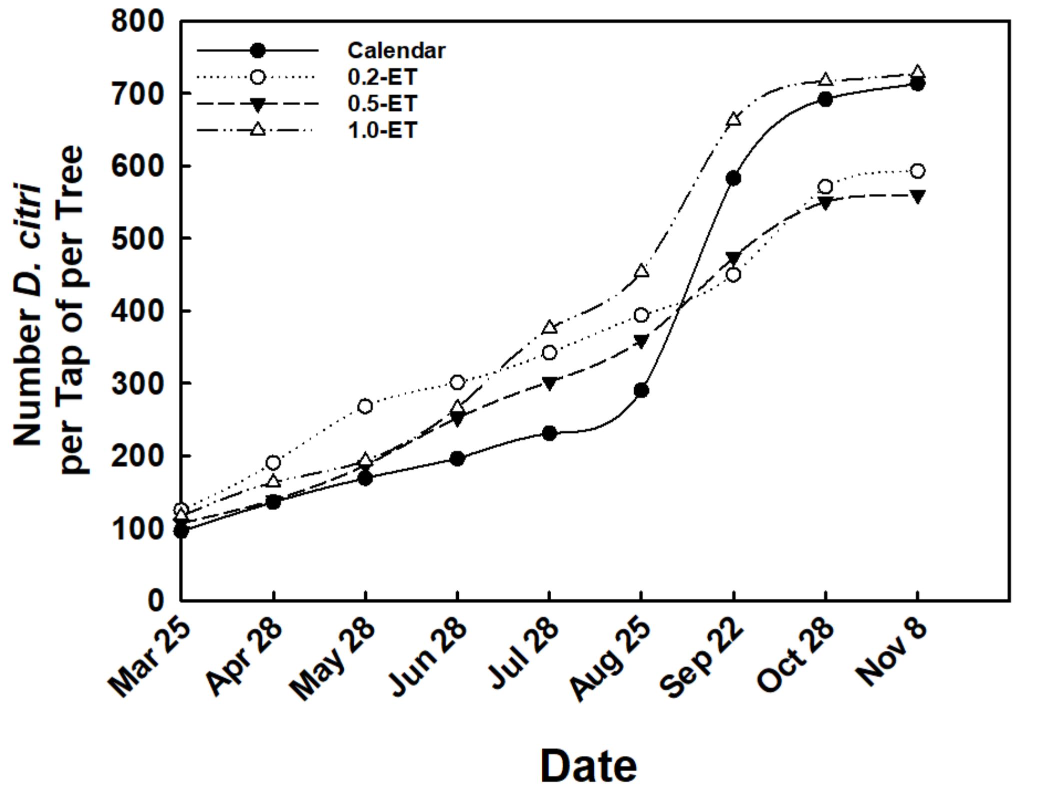
Failure to control ACP adults is coincident with development of neonicotinoid resistance

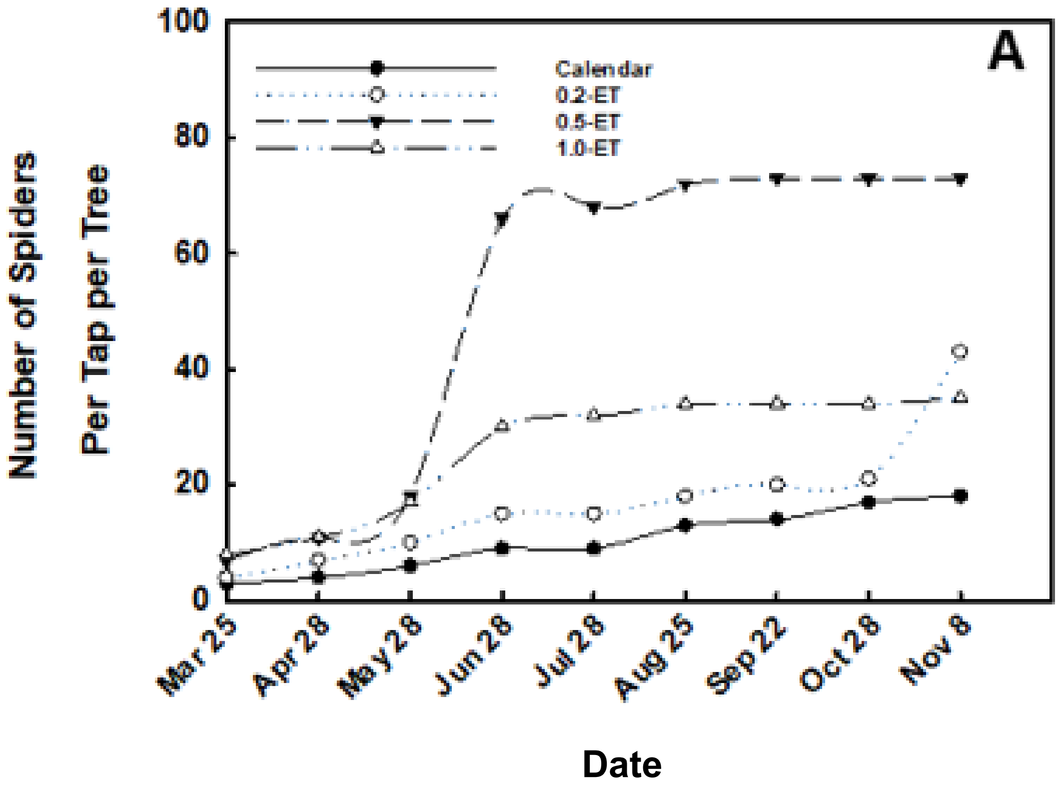
Economic Injury Level of 0.2 ACP/ tap = average of 10 trees sampled



Key Assumptions

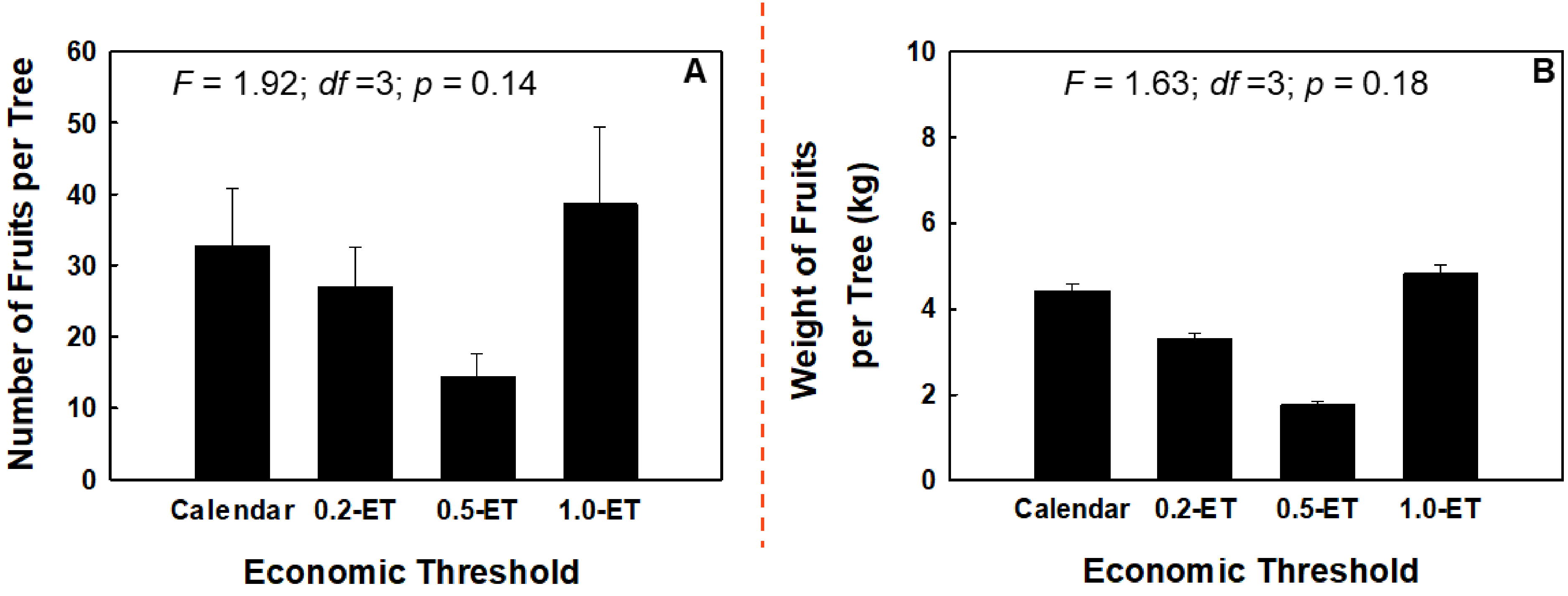
- HLB infection is near 100% and stopping spread is not a goal
- Keeping ACP below the chosen threshold boosts yield via improved tree health





Example

Threshold reduced spray frequency with no negative impact on psyllid counts



Example

Threshold reduced spray frequency with no negative impact on yield

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Estimated management costs ($/ha) and yield losses ($/ha) associated with ACP using thresholds.

Management Approach	Initial ACP	No. sprays	K season[a]	Management costs ($/ha) 1 ha = 2.5 ac	Estimated Yield loss ($/ha)[b] 1 ha = 2.5 ac	Total costs ($/ha) 1 ha = 2.5 ac
Calendar	43	8	714	487.22	271.48	758.70
ET-0.2	53	7	593	451.50	206.24	657.74
ET-0.5	35	4	560	229.0	212.79	441.79
ET-1.0	48	3	728	166.92	236.18	403.09

[a] Cumulative number of *D. citri* for the year.

[b] Due to herbivore damage.

Source: Chen, X. D., D. Stockton, H. Gossett, J.A. Qureshi, F. Ibanez, K.S. Pelz-Stelinski, and L.L. Stelinski. 2022. Comparisons of economic thresholds for Asian citrus psyllid management suggest a revised approach to reduce management costs and improve yield. *Frontiers in Sustainable Food Systems.* 6: 948278.

Cost is the monetary value expressed in $/ha associated with the yield loss resulting from the seasonal number of vectors during Kseason and where P is the orange juice price paid at the harvest, expressed in $/kg of solids.

Novel mechanisms for reducing pathogen transmission are needed

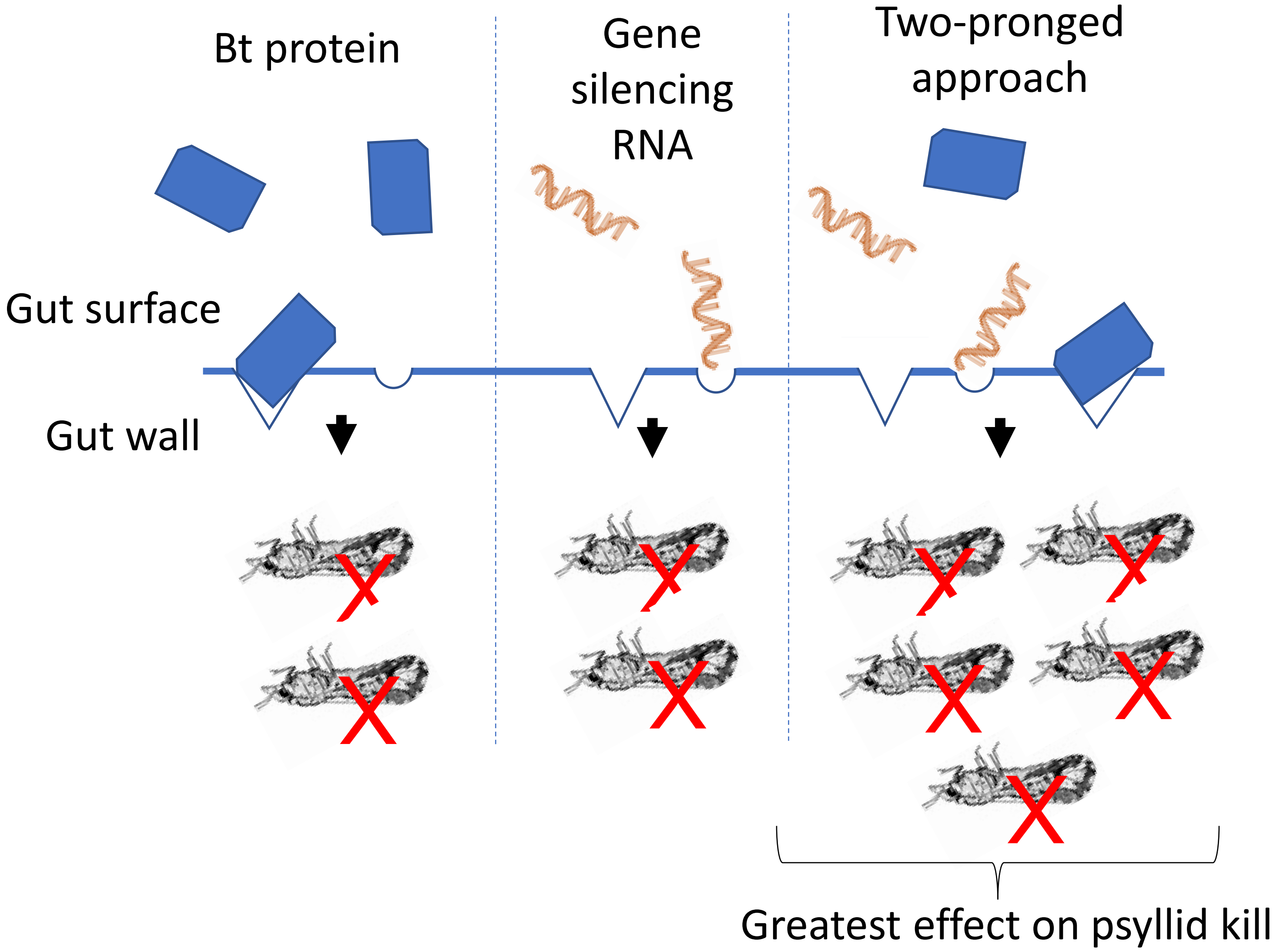
Current management of psyllids with broad spectrum insecticides is unsustainable

- High cost
- Insecticide resistance

Can we manipulate the host or vector to reduce pathogen transmission?

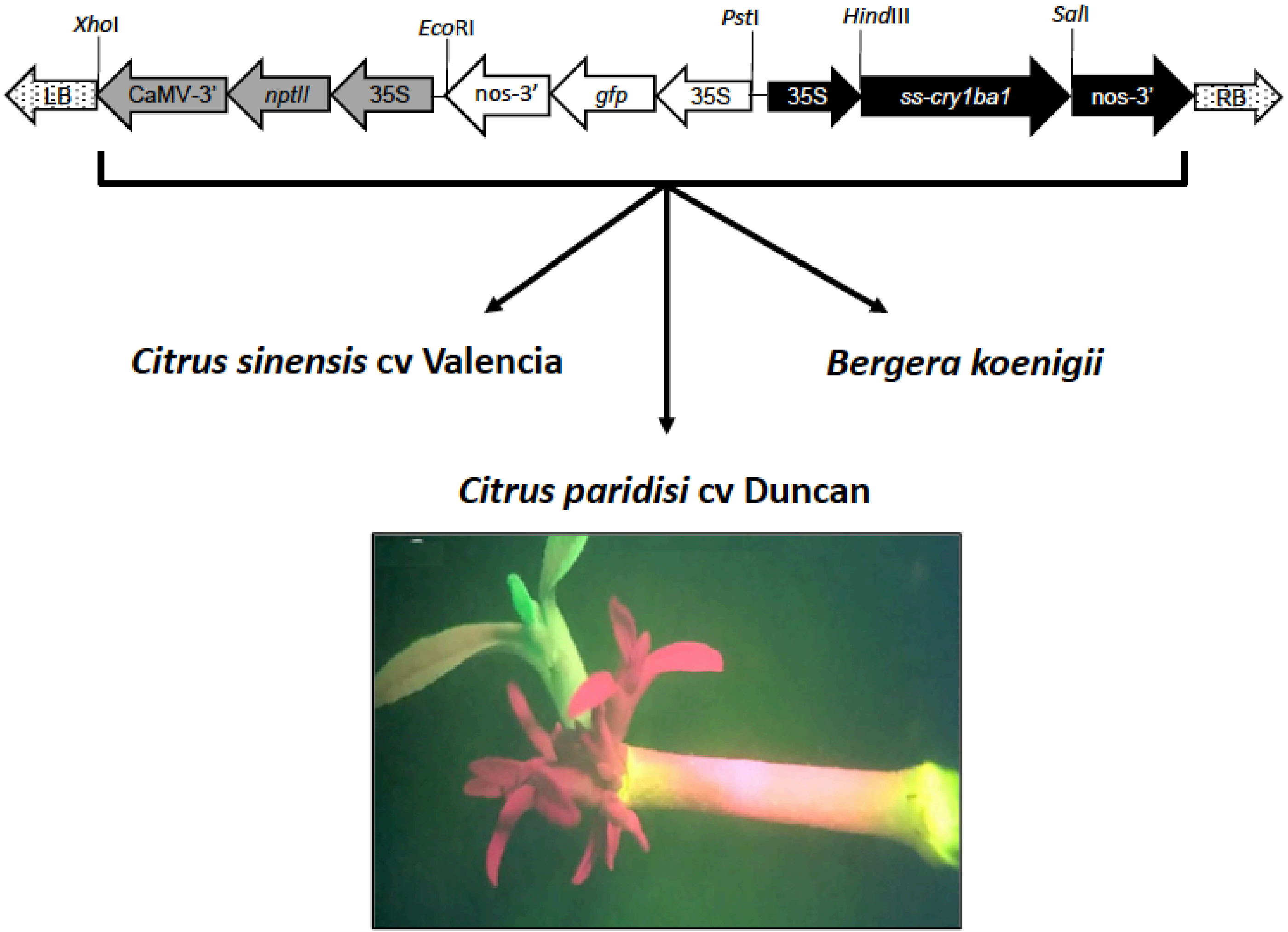
- Manipulation of tree for increased self defense
- Disruption of endosymbionts in vector for HLB management

Manipulate the tree host: Bt protein + gene silencing RNAs

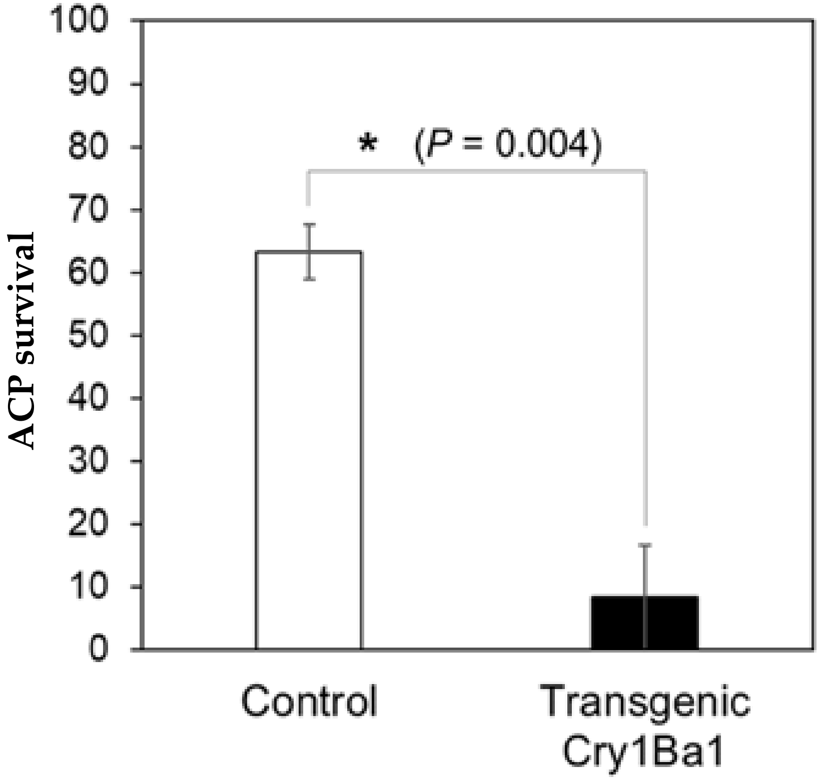


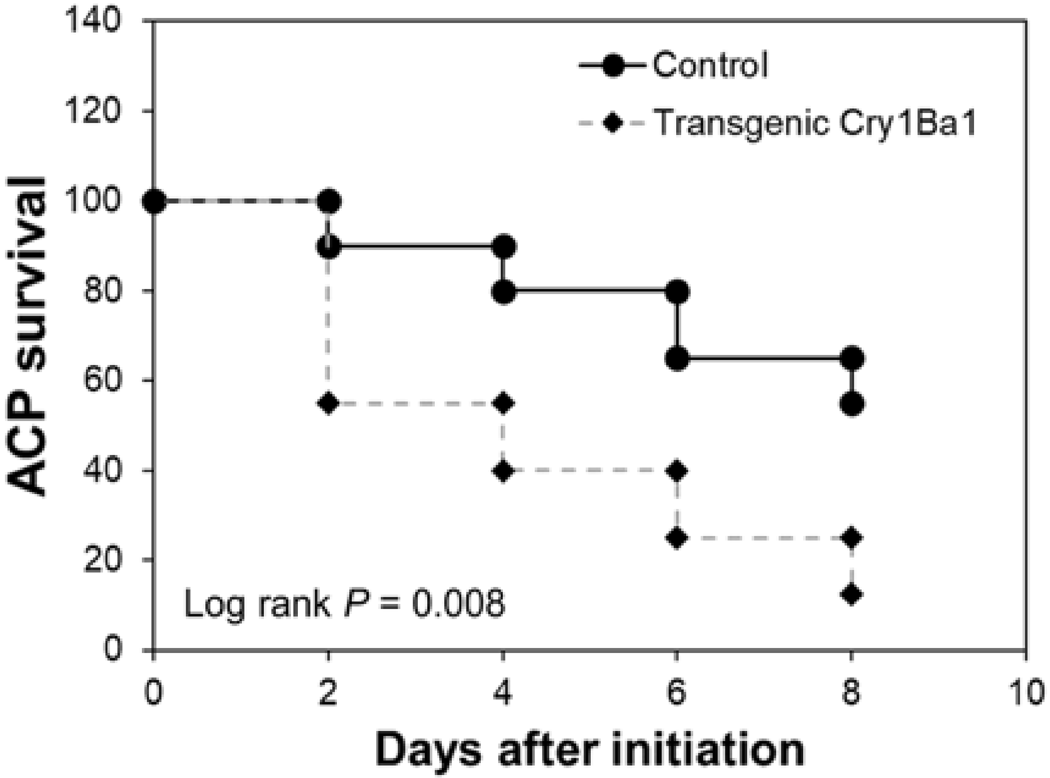
- Schematic of T-DNA used for transformation: The CaMV 35S promoter was used for constitutive transcription of *cry1ba1*.

- An explant with a transgenic shoot of *C. paridisi* cv Duncan with green fluorescence is shown alongside red, non-transgenic shoots.



ACP survival reduced on transgenic plants expressing Cry1Ba1

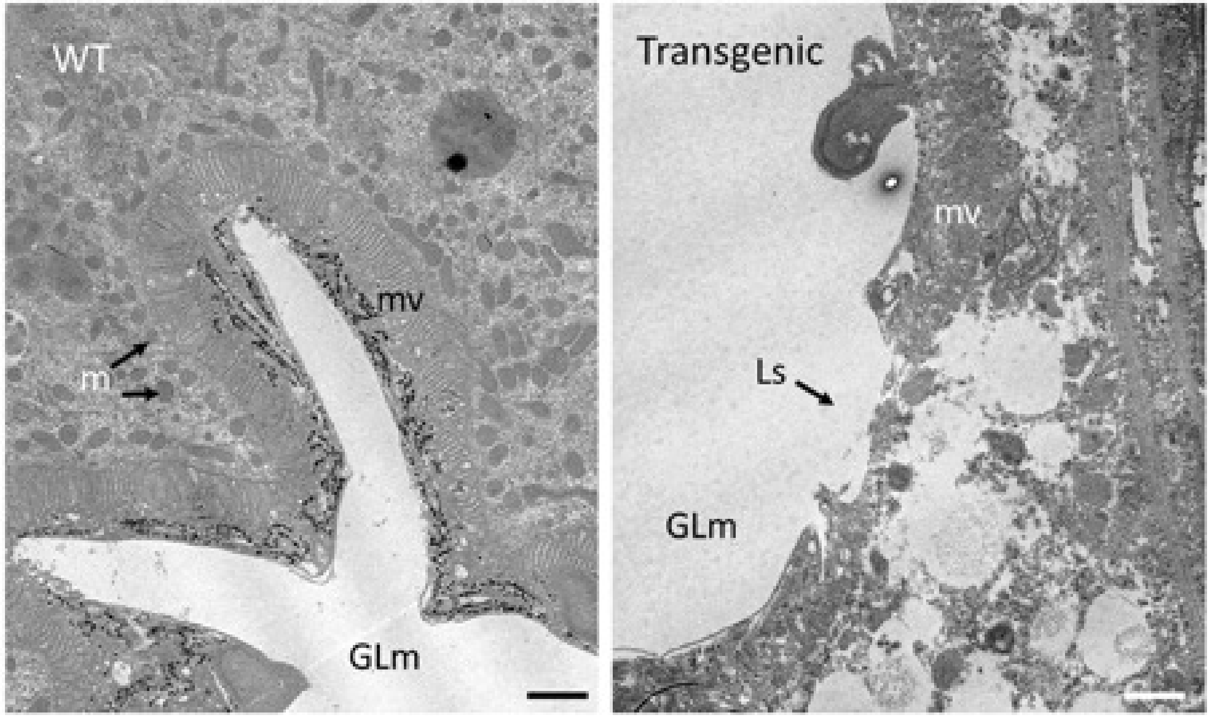




Source: Ravanfar et al. 2022. Genetic modification of *Bergera koenigii* for expression of the bacterial pesticidal protein Cry1Ba1. *Frontiers in Plant Science.* 13: 899624.

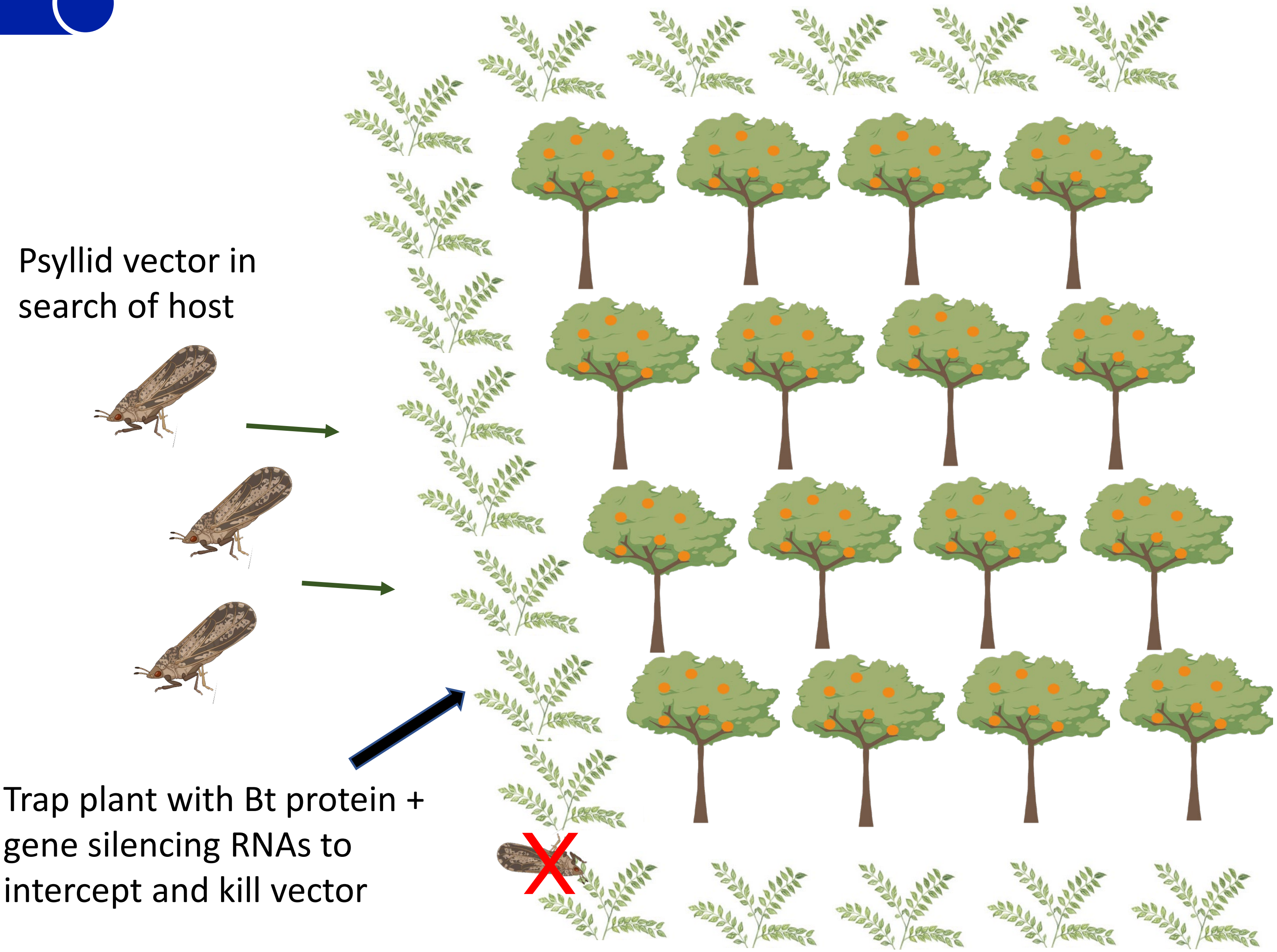
ACP survival reduced on transgenic plants expressing Cry1Ba1

TEM of Cry1Ba1-mediated damage to gut epithelial tissues of Asian citrus psyllid. The intact microvillar lining of the gut epithelium is evident in ACP fed on WT plants. In contrast, the microvilli of insects fed on Cry1Ba1-expressing *B. koenigii* (Transgenic) were sparse and disrupted with multiple lesions apparent.

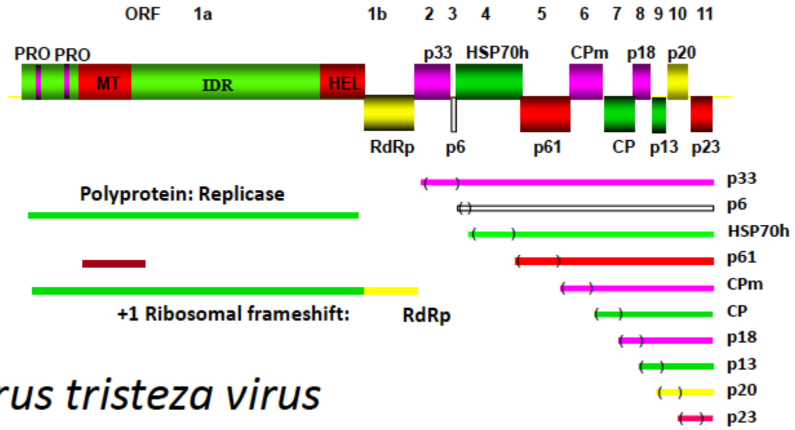


Source: Ravanfar et al. 2022. Genetic modification of *Bergera koenigii* for expression of the bacterial pesticidal protein Cry1Ba1. *Frontiers in Plant Science.* 13: 899624.

Hyper-attractive trap crops expressing active ingredients to intercept and kill the mobile vectors obviate the need for transgenic crop

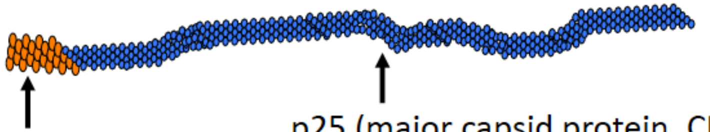


CTV-mediated expression of Bt toxins-A non-transgenic plant delivery option for delivery of toxins to target ACP

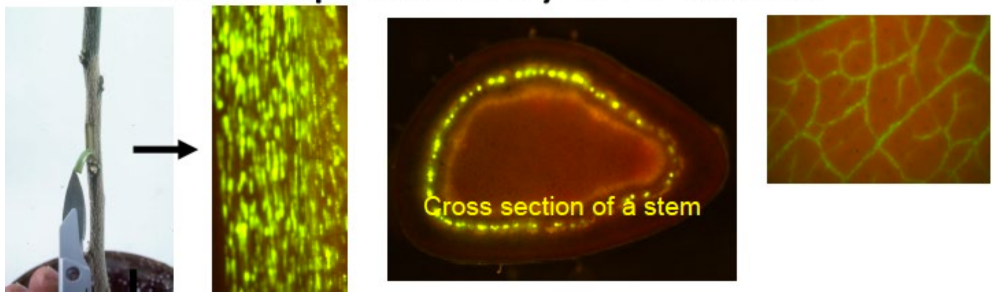


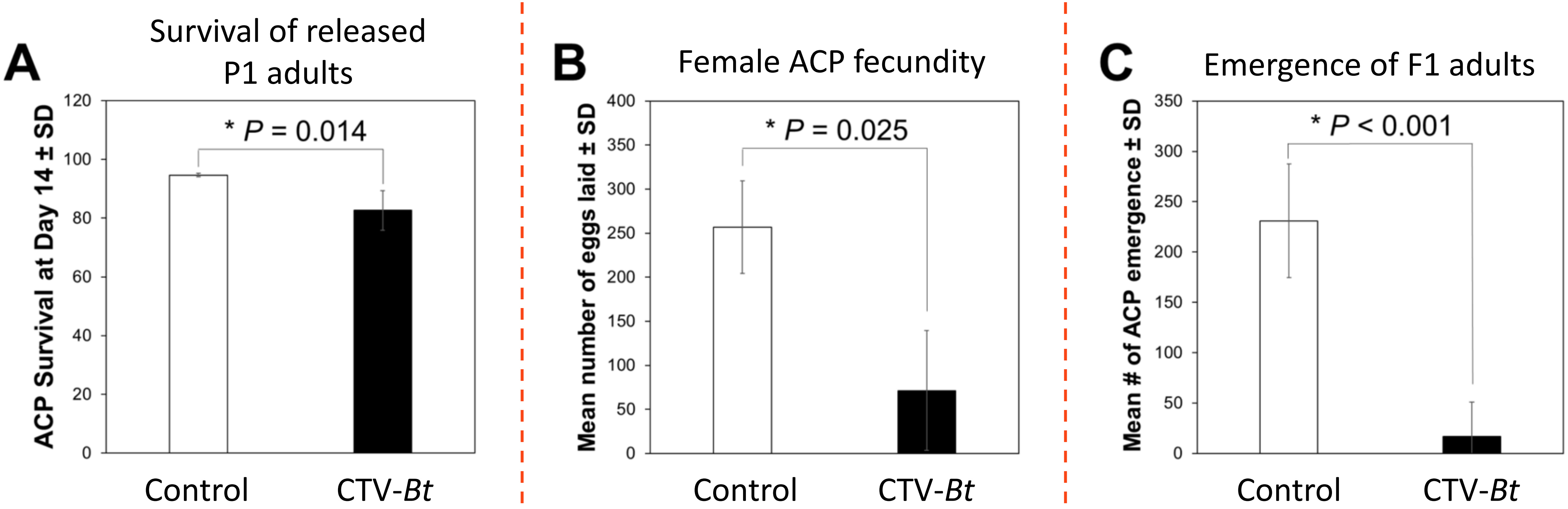
Citrus tristeza virus

CTV virion



GFP Expressed by CTV vector





- Fecundity of ACP females feeding on *CTV-Bt* is reduced
- Emergence of the subsequent generation is also reduced

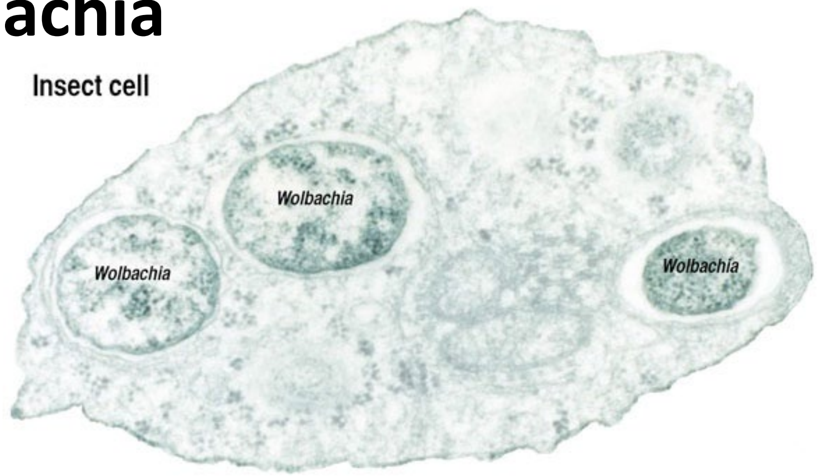
Manipulate the vector: Reduce transmission by targeting ACP gut

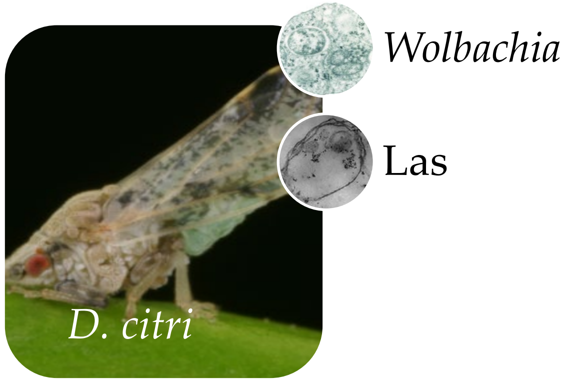
- A widespread intracellular bacterium, carried by an estimated 40% of insect spp.

- May interact with pathogens, affecting the probability of transmission (e.g. competitive exclusion, immune activation)

- Approach used in insect transmitted human pathosystems

Wolbachia



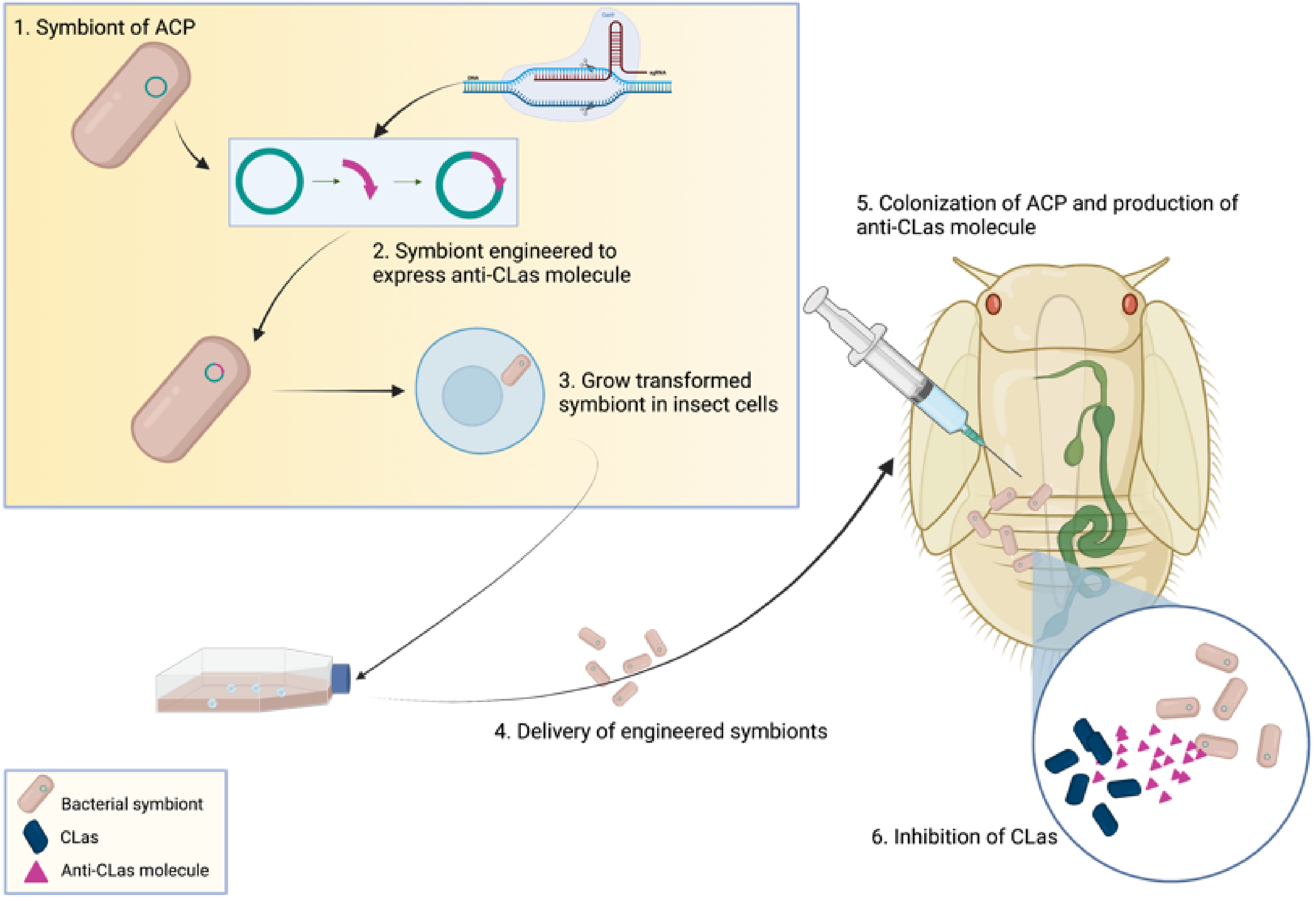


Symbiont-mediated control

- Paratransgenesis: Introduce a phenotype-altering transgene into the target vector populations using commensal symbionts as the vehicle for incorporating the transgene into ACP

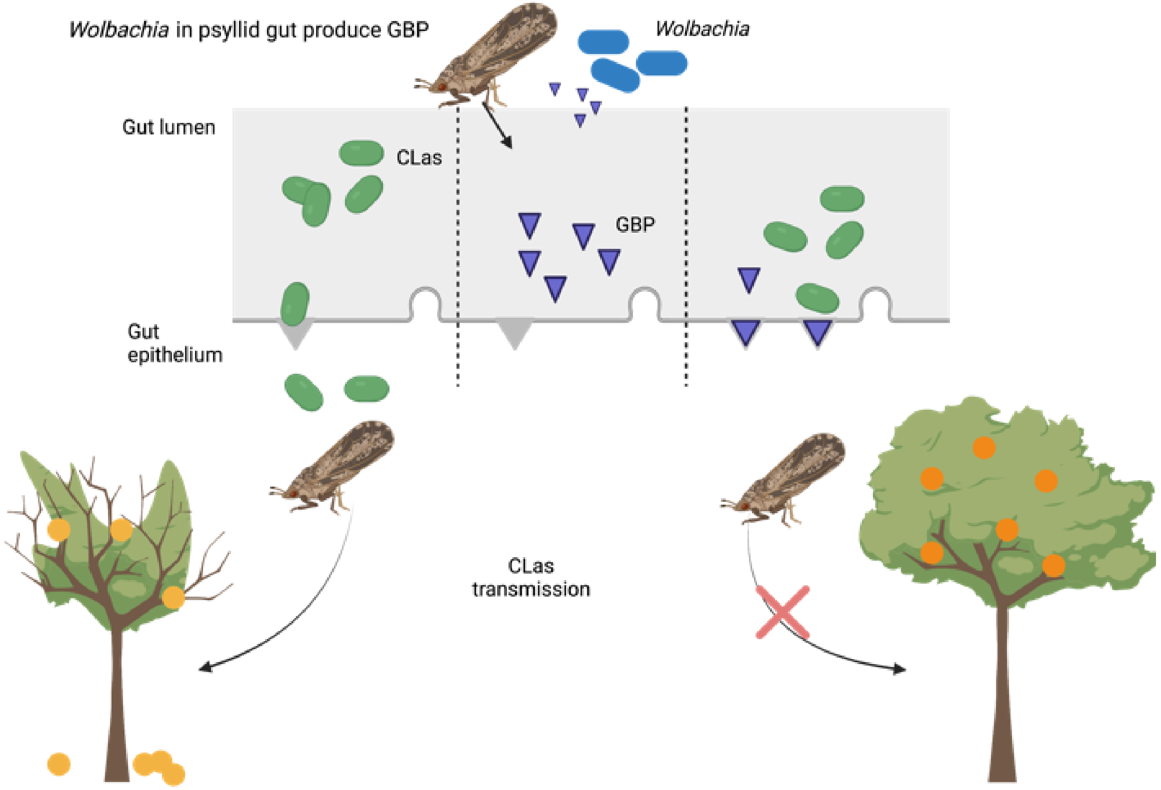
- Ultimately, the desired phenotype is one that would disrupt CLas transmission

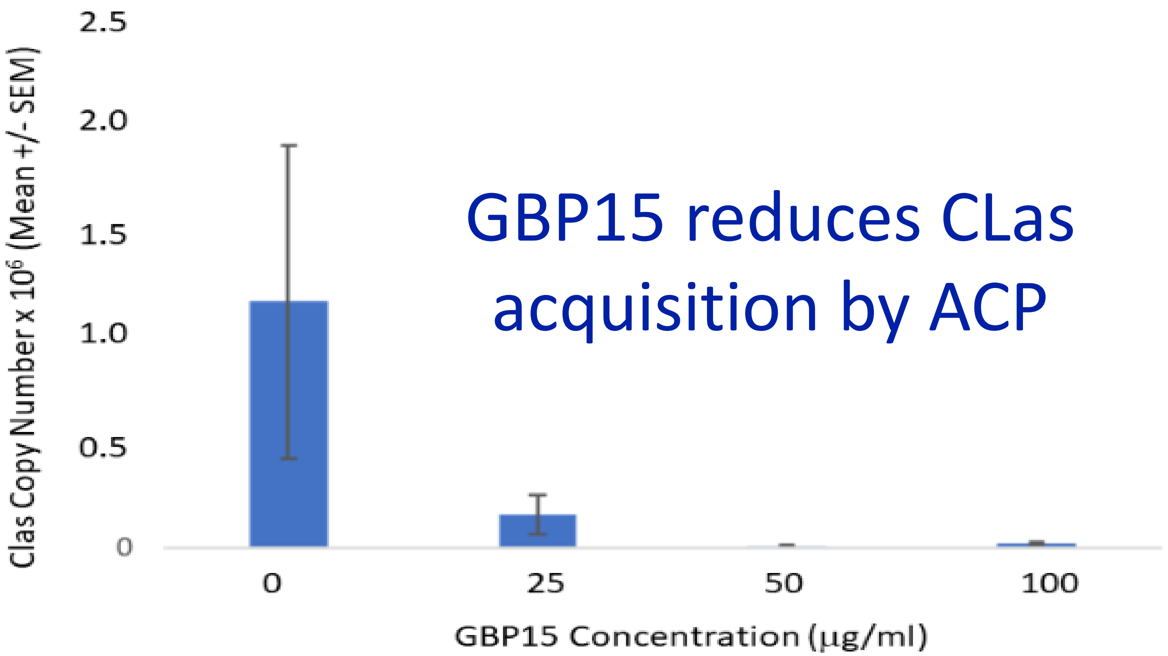
Reduce transmission by targeting CLas and ACP gut



Source: Pelz-Stelinski et al.. 2019. Genetically modified Wolbachia for biocontrol of insect bacterial disease vectors. US PAT.61094.

Wolbachia as a delivery system for CLas-blocking peptides or silencing RNAs





GBP15 reduces CLas acquisition by ACP

New biotechnological approaches to manage HLB

- A new alternative for suppressing ACP populations is the use of Bt pesticidal proteins produced by bacteria.
- One approach is to engineer the actual plant to produce the ACP-killing Bt protein in its phloem.
- We envision creating 'trap plants' that are more attractive to psyllids than cultivated citrus.
- A second approach is to use a plant virus (Citrus tristeza virus) that replicates in citrus phloem as a delivery vehicle for Bt.
- Gene silencing RNAs that reduce or block the expression of genes that psyllids need for survival show promise as control tools.
- One novel approach for developing non-transmitting ACP is to modify endosymbionts (*Wolbachia*) living in psyllids to produce novel proteins that target or block the CLas pathogen.
- This process of paratransgenesis allows us to create a psyllid with new characteristics because modified endosymbionts that are re-introduced into the insect can produce new proteins.

Managing psyllids while reducing cost

- Psyllid density is related to tree stress—more psyllids--> higher damage, which compromises tree health (yield)
- Spray for adults at bud break at the beginning of first flush before there is feather flush on which adults can lay eggs.
- If the pest population (and the resulting damage) is sufficiently low, it might not pay to take control measures
- As the pest population continues to rise, it reaches a point where the resulting damage (=reduced immune response) would justify taking control measures
- 0.2 – 1.0 psyllids per tap seems like an effective ballpark.

Thank you!