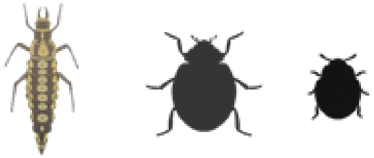
Use of Predators Against *Diaphorina citri* in Residential Buffer Zones

Area-wide ACP program

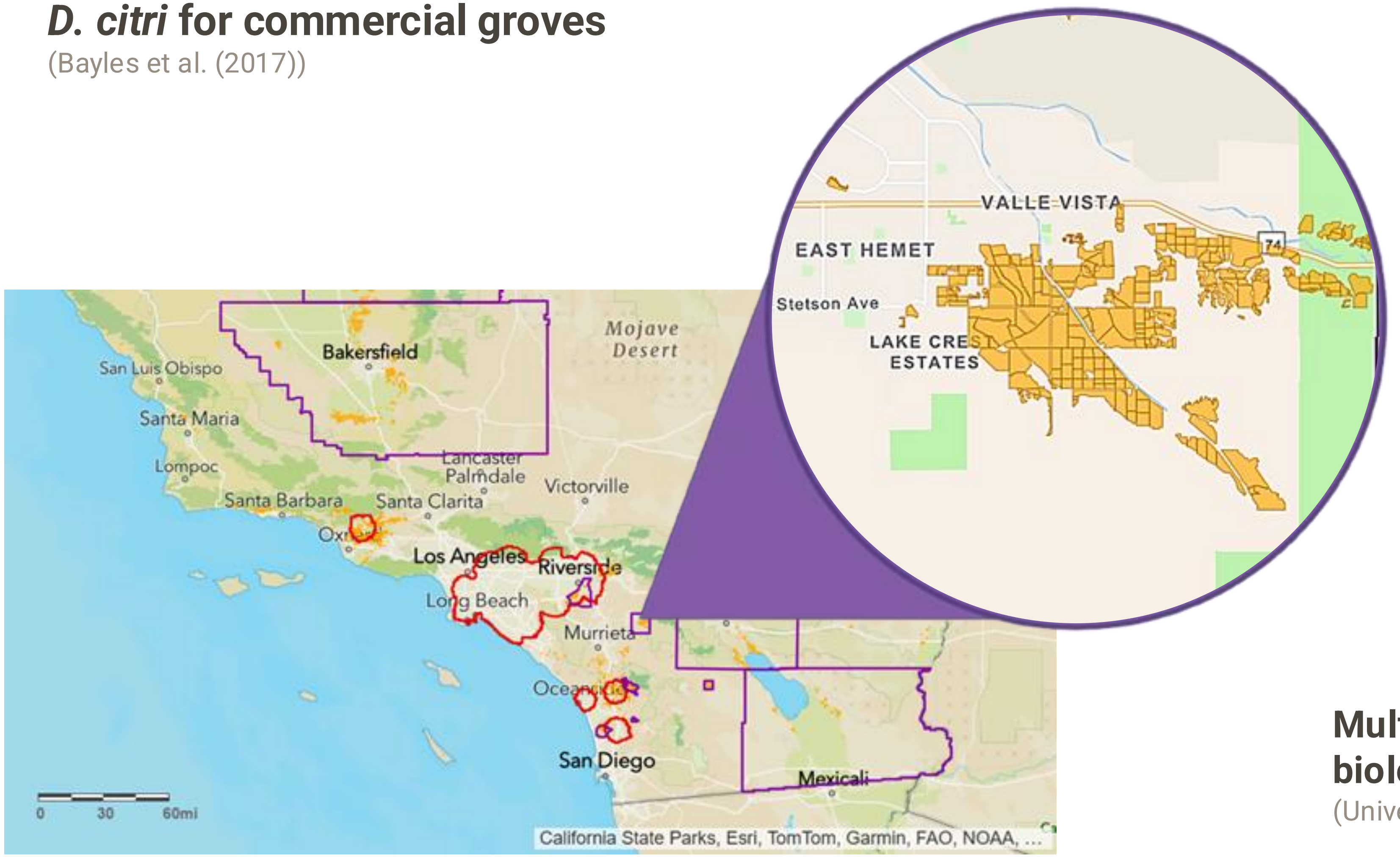
Use of biological control in the residential-commercial buffer zone

- Multi-year project
- Multiple pests
- Ant control
- Predators ← Preliminary results from 1st year



Evaluate mass release of beneficial predators for citrus pest control in residential trees

Residential citrus can be the main source of *D. citri* for commercial groves
(Bayles et al. (2017))



Multiple effective predators identified as biological control candidates
(University of California, 2021; Gómez-Marco et al., 2022)



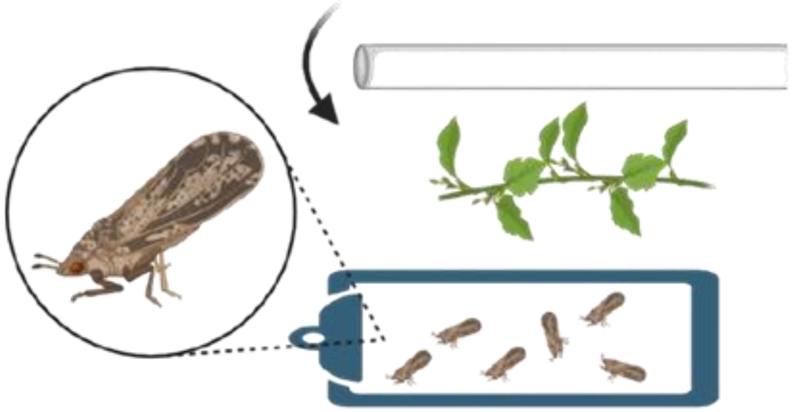
We are releasing 3 predators every 2 weeks:

 **1.** ***Diomus pumilio***	 2 mm (Aguilar et al., 2007)	Not reared commercially 25/tree	Each individual may consume up to **~120-130** soft-bodied insect prey (Aguilar et al., 2007)
 **2.** ***Rhyzobius lophanthae***	 3 mm (Mike Quinn, 2025)	Commercially available 50/tree	Each individual may consume **~100-200** soft-bodied insect prey (Quesada and Sadof, 2020; UC ANR, 2025)
 **3.** ***Chrysoperla rifilabris***	 6-12 mm (J. García-Quiceno, 2025)	Commercially available 50/tree	Each individual may consume **more than 200/week** soft-bodied insect prey (Carillo et al., 2025)

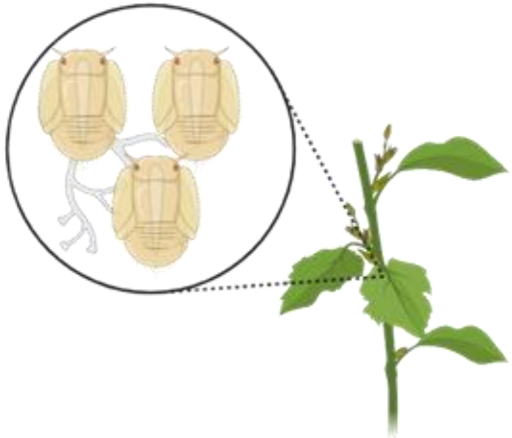
Pests sampled every month:

32 residential sites randomly assigned (8 replicates per treatment).

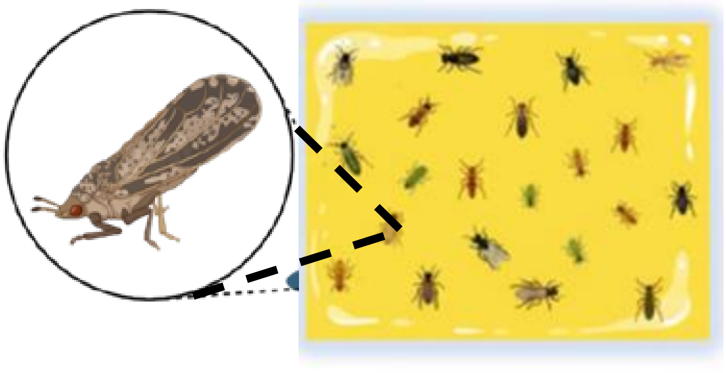
Tap sampling



Visual sampling

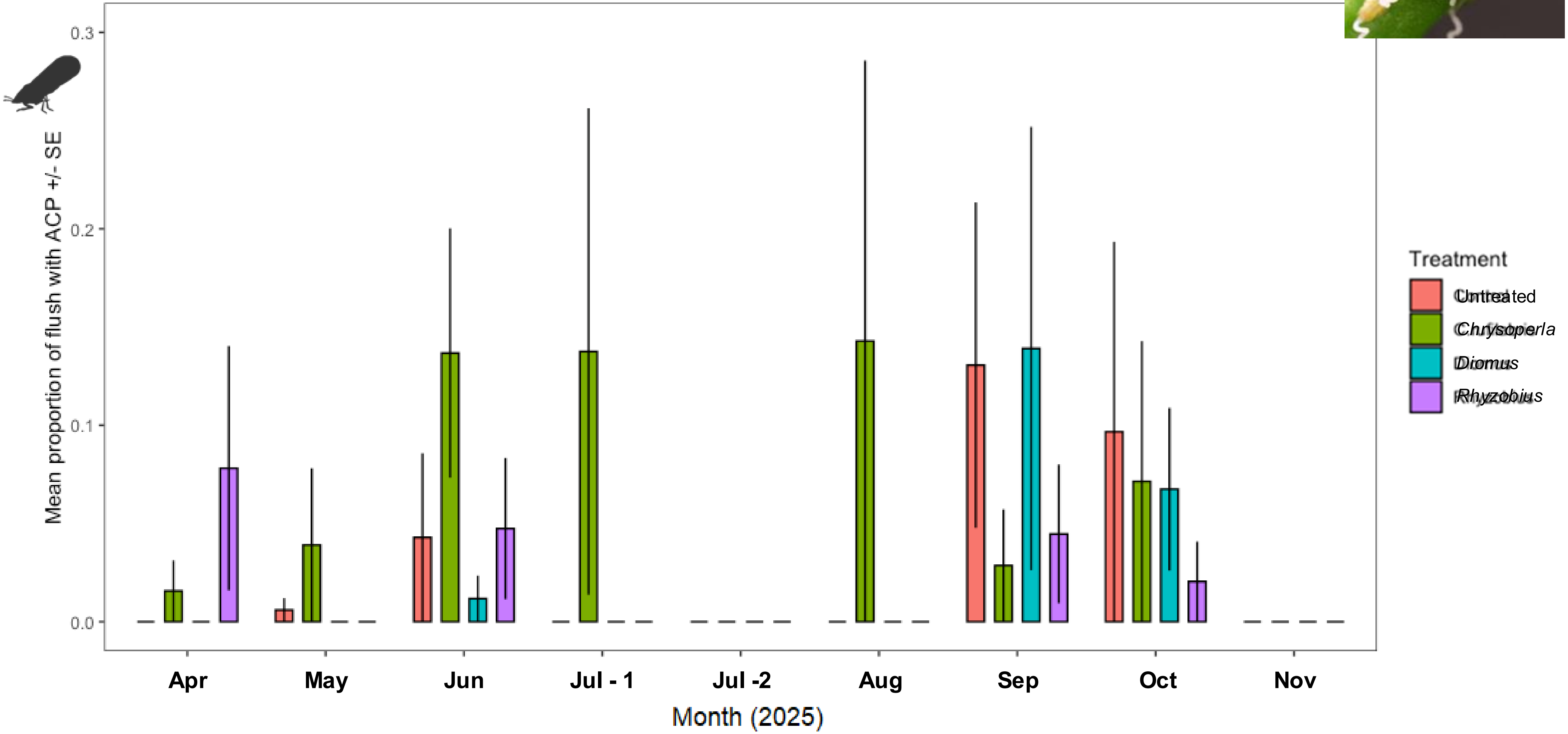


Yellow sticky trap



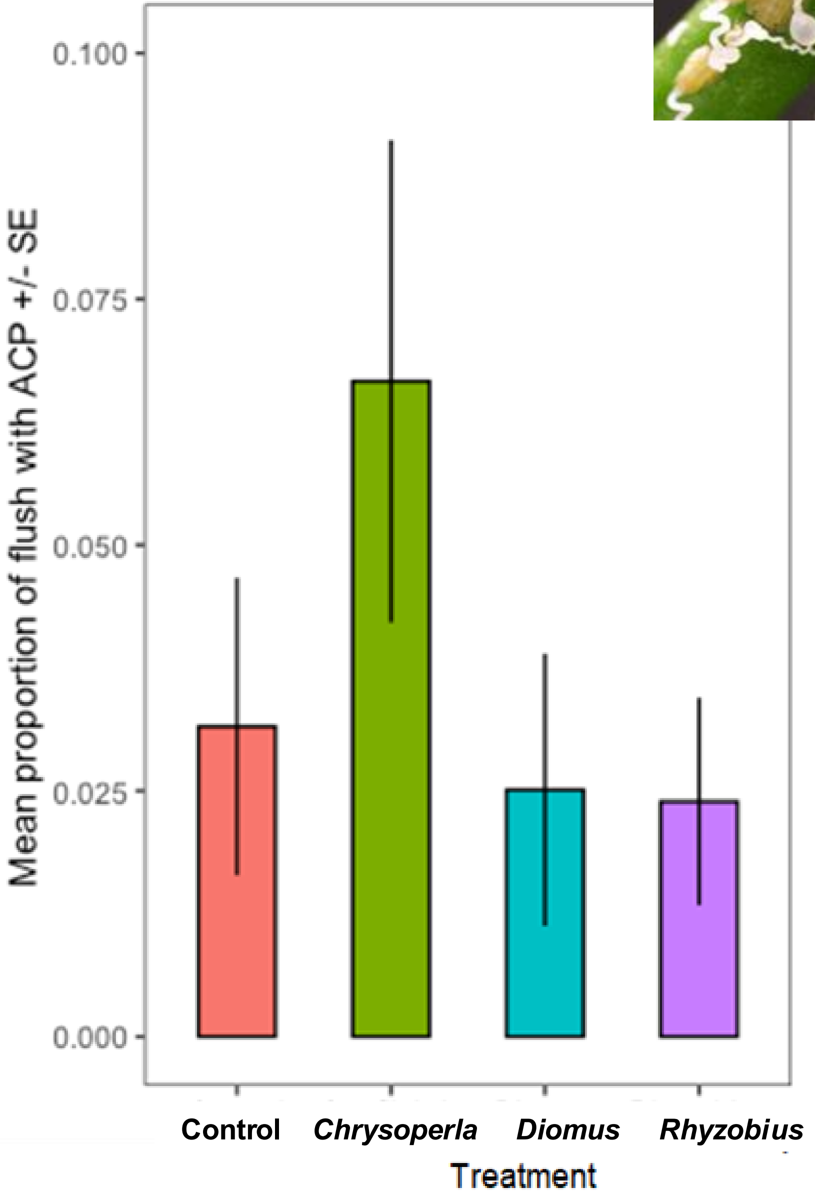
Week	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6
Application	Y	N	Y	N	Y	N
Sampling	Tap+Visual		Tap		Visual	
Sampling Sticky	Put out				Collect and put out a new one	

Results: Visual sampling /Flush Points – ACP Nymphs

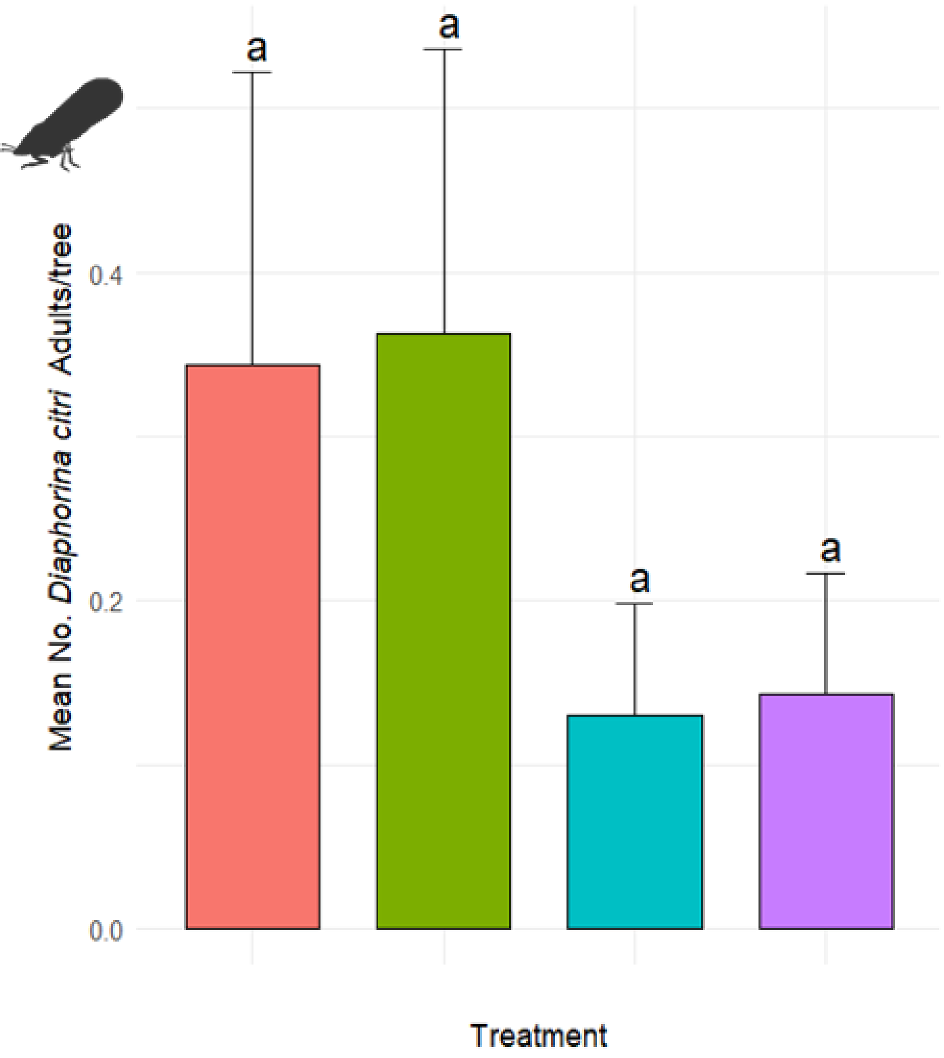


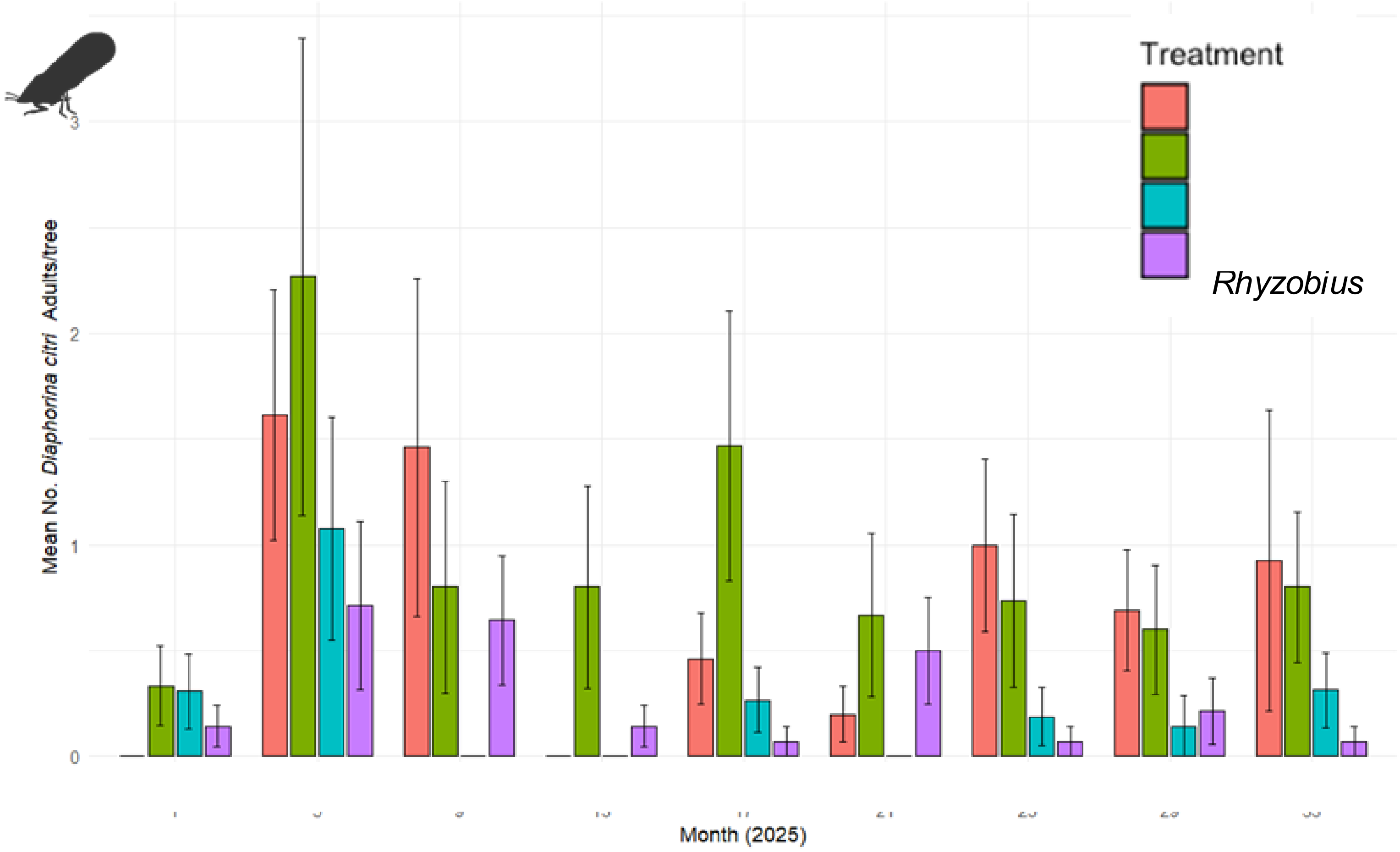
Results: Visual sampling /Flush Points – ACP Nymphs

```
glmmTMB(countACP~ Treatment + Week + (1|Block/Site),
                      ziformula = ~1 + (1|Block/Site),
                      family = nbinom2)


Conditional model:
                     Estimate Std. Error z value Pr(>|z|)
(Intercept)           0.03714    1.00268   0.037   0.9705
TreatmentC.rufilabris 1.21248    0.72252   1.678   0.0933 .
TreatmentDiomus      -0.03547    0.66913  -0.053   0.9577
TreatmentRhyzobius    0.54207    0.77743   0.697   0.4856
Week                  0.01177    0.03044   0.387   0.6990
---


Zero-inflation model:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   2.4541     0.7182   3.417 0.000634 ***
```

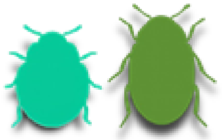


Results: Tap sampling – ACP Adults





D. pumilio and ***R. lophanthae*** appear to be **promising** biological control agents against **ACP**

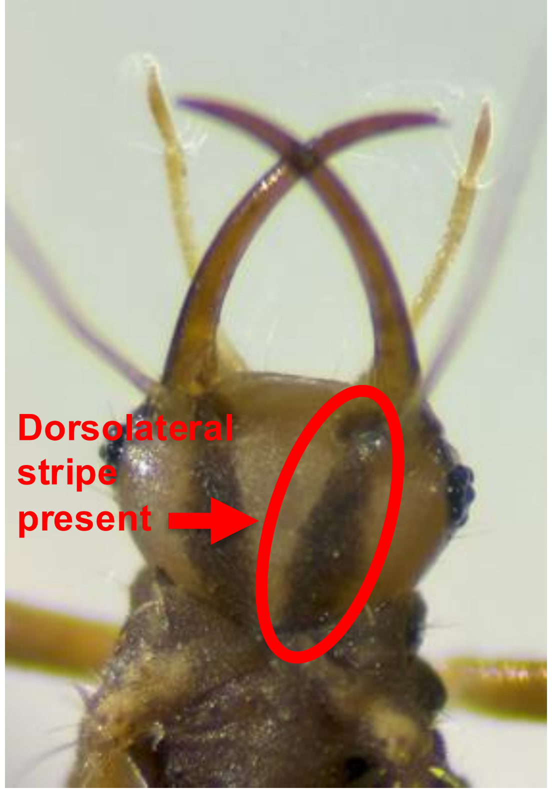


The treatment of **lacewings is not looking promising** for controlling **ACP** in this experiment

Cryptic species: *C. comanche* is what we find in citrus grove surveys

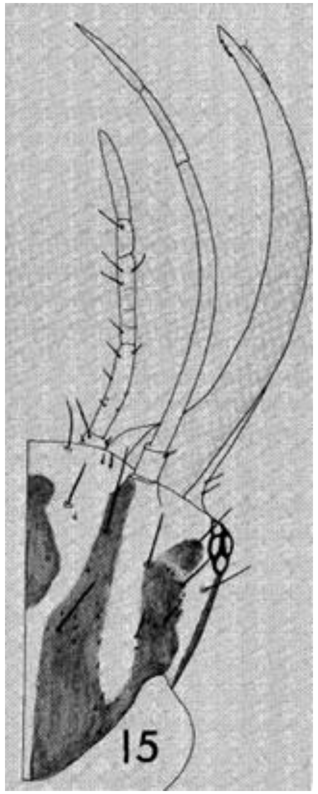
(Gómez-Marco et al., 2022)

Larval morphology



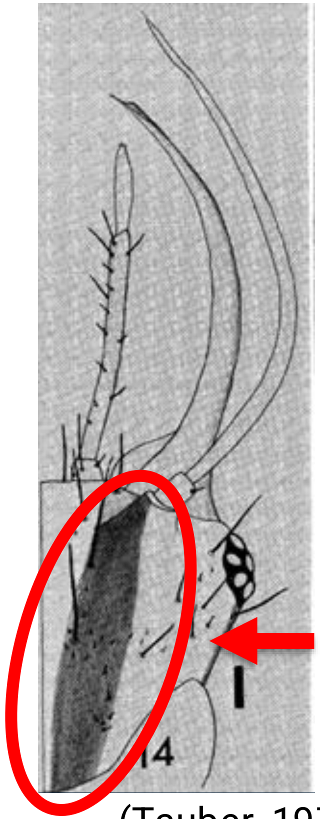
(J. García)

Chrysoperla rufilabris
Insectary



(Tauber, 1974)

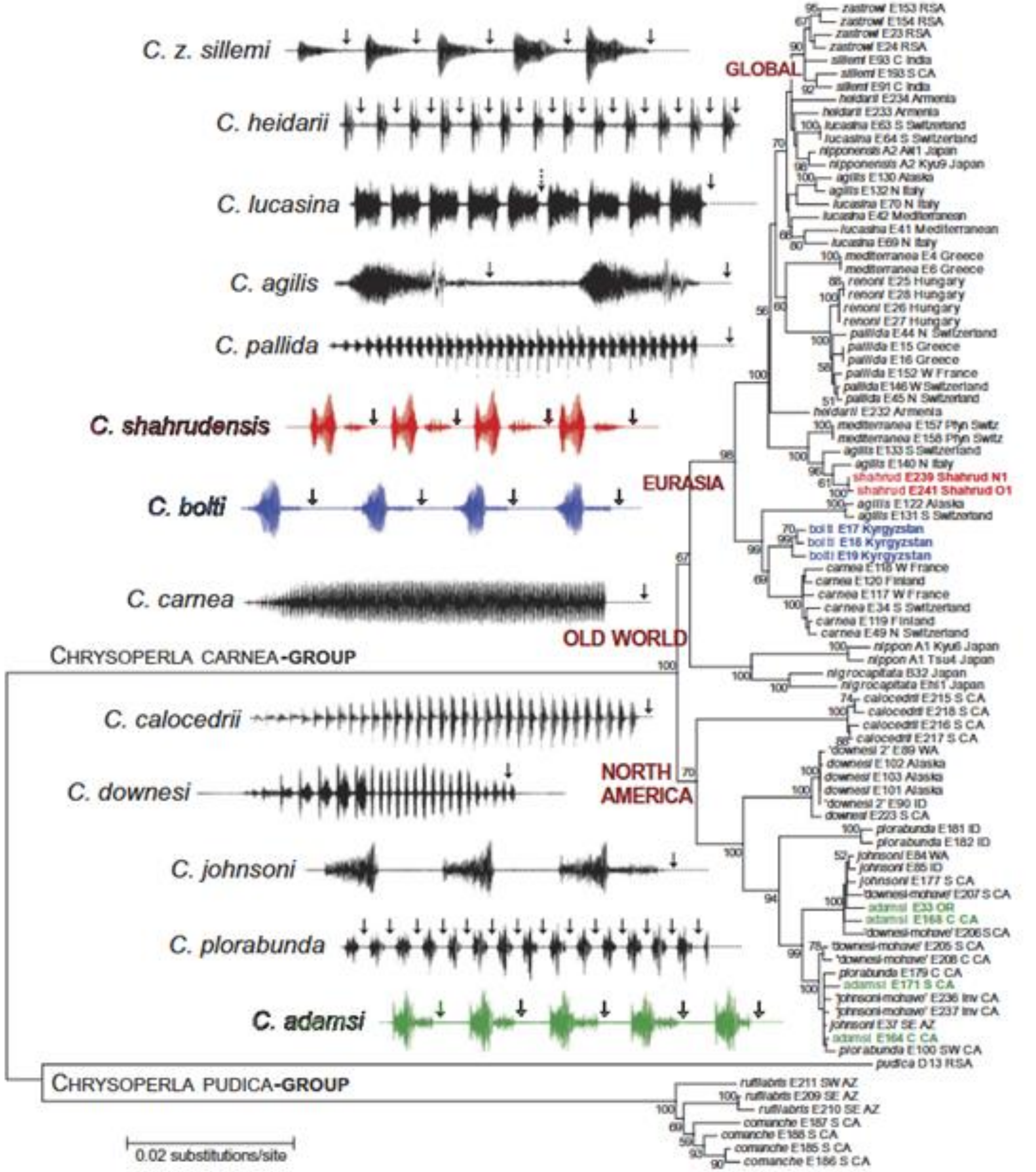
Chrysoperla comanche



(Tauber, 1974)

Chrysoperla rufilabris

Courtship songs *Chrysoperla* spp.

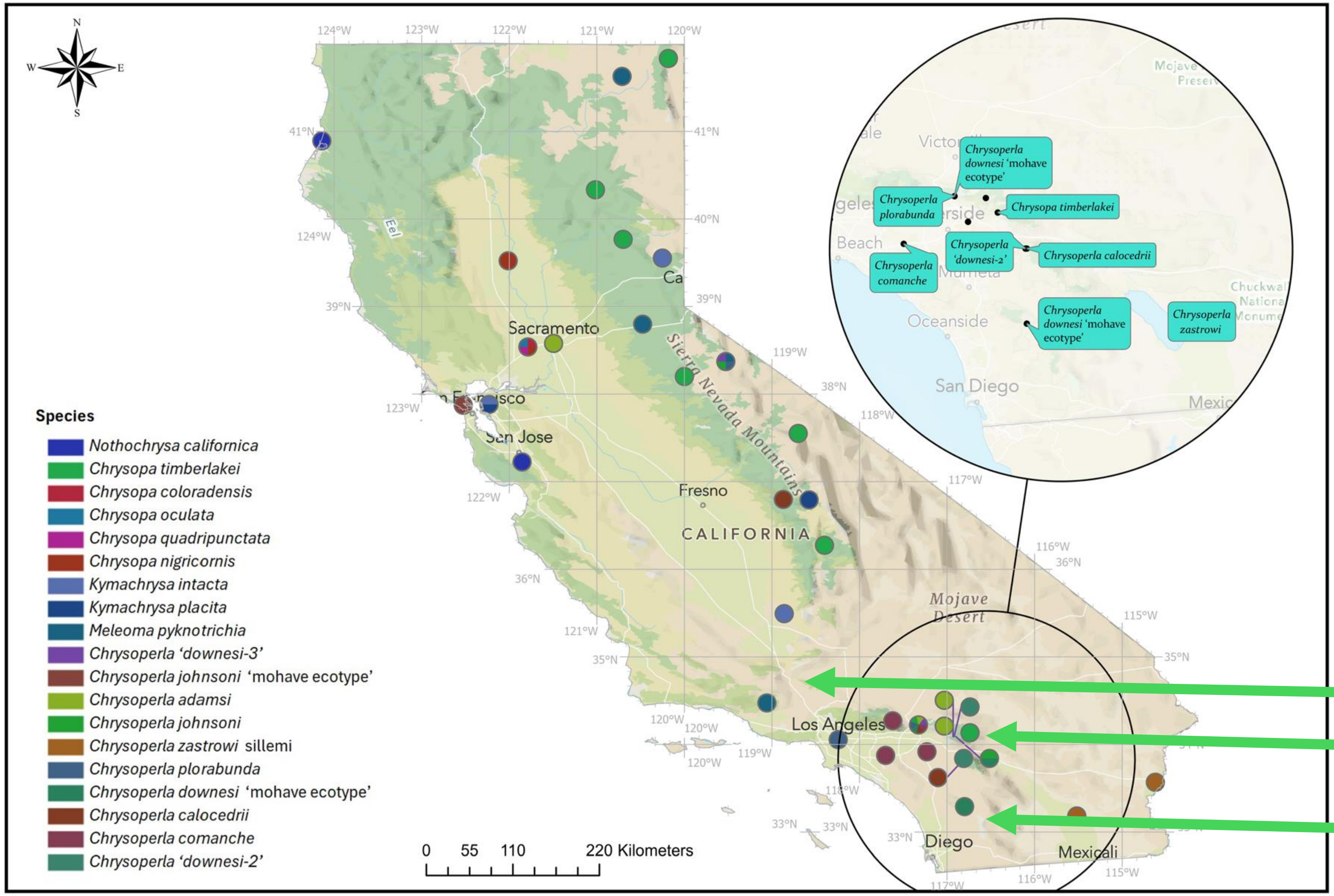


(Tauber, 1974)

Courtship songs *Chrysoperla* spp.

Historical lacewing records

Geographic Distribution of Chrysopidae (Green Lacewings) in California



Current phenological sampling

Monthly DVac

10 trees on edges of 3 commercial groves per 3 regions

~2.5 adult lacewings per site month

Development of a Colony of Wild *Chrysoperla comanche*

(J. García, 2025)

- Field collections
- Identification
- Obtaining eggs and larvae
- Well plates as cages
- Provide diet: honey–water or yeast
- Obtaining adults
- Ship to commercial insectary

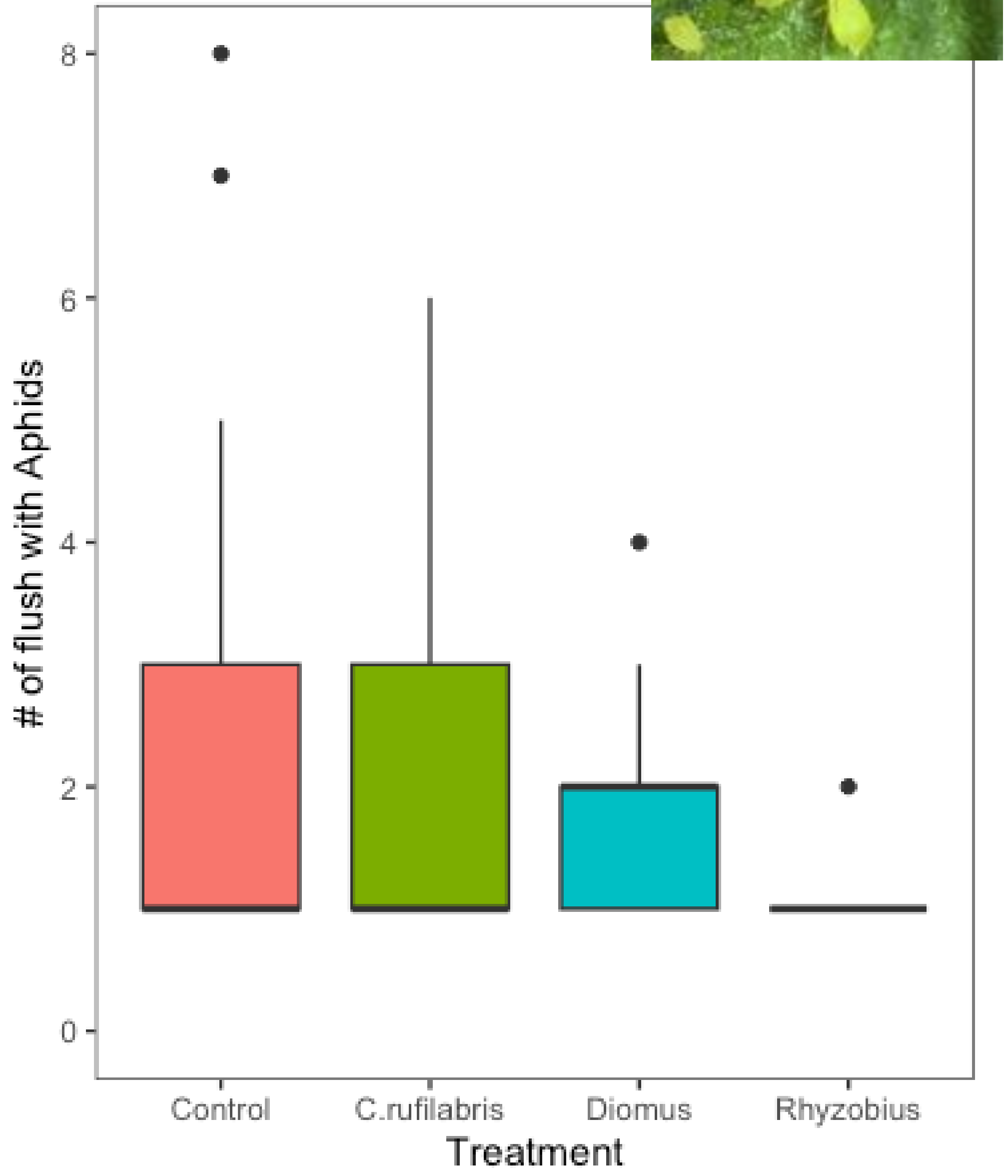
Pest Insects	Beneficial insects and others
Visual sampling: flush points	
ACP nymphs	Predators
Aphids	Spiders
Visual sampling: Inner Branches	
Brown scale	Predators
Citrus mealybug	Spiders
Red scale	
Long tailed mealybug	
Visual sampling: Mature fruit	
Brown scale	Predators
Citrus mealybug	Spiders
Red scale	
Visual sampling: Young fruit	
Damaged fruit: thrips/lep	Spiders

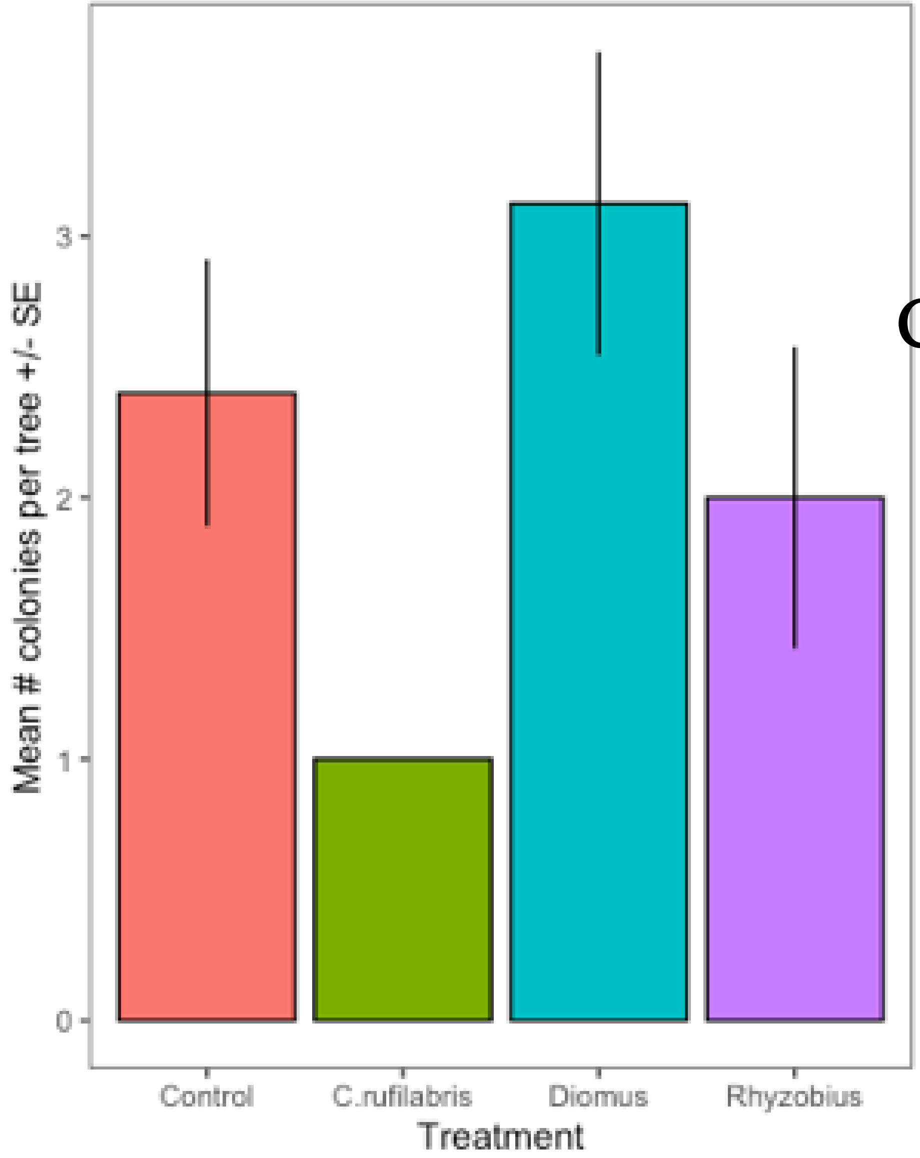
Pest Insects	Beneficial insects and others
Tap sampling	
ACP nymphs	Lady beetles
Aphids	Green lacewings
Ants	Spiders

Pest Insects	Beneficial insects and others
Sticky traps	
ACP adults	Lady beetles
Sharpshooters	Green lacewings
Ants	Spiders
Thrips	

Results: Visual sampling

Aphids





Citrus Mealybug

Conditional model: Estimate Std. Error z value Pr(>|z|)

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	0.5936	0.3794	1.565	0.1176	
TreatmentC.rufilabris	-1.9735	0.8338	-2.367	0.0179	*
TreatmentDiomus	0.5096	0.4309	1.183	0.2369	

Plan 2026

Hemet Project Checklist

Biocontrol Project (every two weeks):

Monday

- € Prepare and print data sheets/maps/checklists, staff coordination for the week
- € Count out and fully label sticky card traps (once a month)
- € Diomus colony and acacia maintenance

Tuesday

- € Receive lacewings, check viability and divvy up into batches for field work
- € Pick up Rhyzobius from vendor and process into tubes for field work (driving required)
- € Collect required number of Diomus and process into tubes for field work
- € Remind homeowners of field work

Wednesday

- € Data collection at sites, tap sampling 2-4 trees per site or flush survey (alternate)
- € Remove old sticky card traps and hang new ones on 2-4 trees per site (once a month)
- € Predators released on trees at study sites
- € Area-wide release of predators at general release sites
- € Diomus colony and acacia maintenance

Thursday

- € Data entry from week's field work
- € Data collection at sites, tap sampling of anywhere from 2-4 trees per site
- € Remove old sticky card traps and hang new ones on 2-4 trees per site (once a
- € Predators released on trees at study sites
- € Area-wide release of predators at general release sites

Friday

- € Data entry from week's field work
- € Process sticky cards taken from the field (once a month)
- € Write summary of week's work for reporting
- € Diomus colony and acacia maintenance

Ant Control Project (every two weeks):

Monday

- Prepare and print data sheets/maps/checklists, staff coordination for the week
- Count out and fully label sticky card traps (once a month)
- Prepare cotton balls & create labeled bags for all cotton balls used during the week
- *Diomus* colony and acacia maintenance
- Ship cotton ball sample (weekly) and sticky cards (monthly) to USDA from previous week

Tuesday

- Measure out Antixx materials that will be used for field work (once a month)
- Measure out ISOTRAIL-AA materials that will be used for field work (once every two months)
- Remind homeowners of field work

Wednesday

- Data collection at sites; tap sampling of 2-4 trees per site tap or flush survey (alternate)
- Place cotton balls onto 2-4 trees per site for 30 minutes & then retrieve and bag them
- Remove old sticky card traps and hang new ones on 2-4 trees per site (once a month)
- Apply Antixx around the base of all trees at site (once a month)
- Apply the ISOTRAIL-AA to lower trunks of all trees at site (once every two months)
- *Diomus* colony and acacia maintenance

Thursday

- Data collection at sites; tap sampling or flush survey (alternate) of 2-4 trees per site
- Place cotton balls onto 2-4 trees per site for 30 minutes & then retrieve and bag them
- Remove old sticky card traps and hang new ones on 2-4 trees per site (once a month)
- Apply Antixx around the base of all trees at site (once a month)
- Apply the ISOTRAIL-AA to lower trunks of all trees at site (once every two months)

Friday

- Data entry from week's field work
- Process cotton balls retrieved from field sites
- Process sticky cards taken from the field (once a month)
- Write summary of week's work for reporting
- *Diomus* colony and acacia maintenance

UC RIVERSIDE

DEPARTMENT OF ENTOMOLOGY

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Evaluation of Argentine ant control for area-wide control of Asian citrus psyllid (ACP).

R. Pandey (CRB), R. Henderson & G. Simmons (USDA)

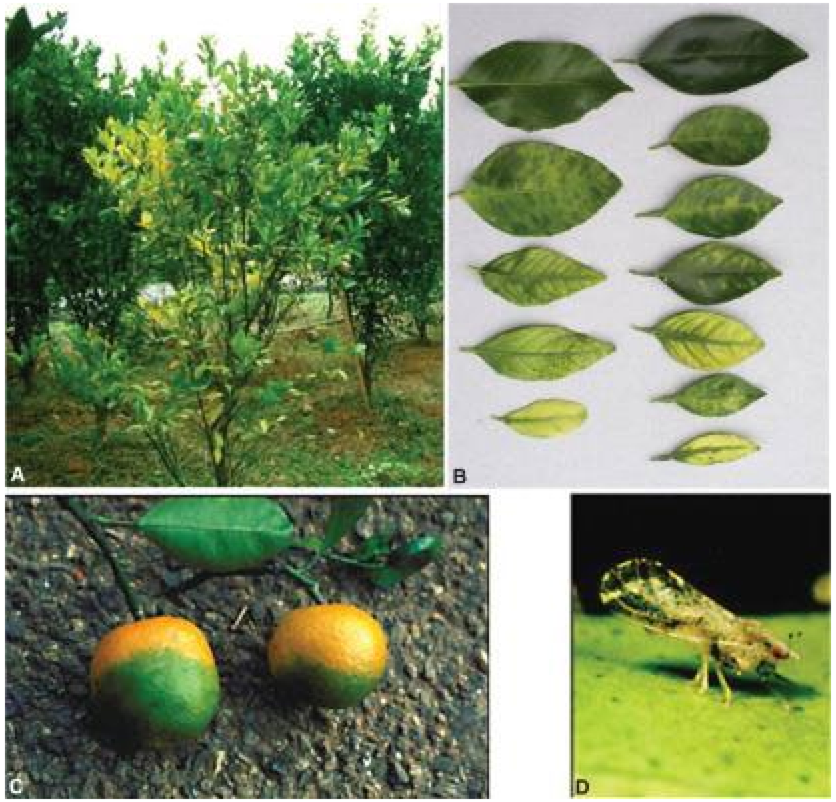


Photo Credit Science Direct

Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama

- Vector of *Candidatus* Liberibacter asiaticus, causal agent of huanglongbing (HLB), citrus greening disease
- Citrus greening (HLB) major citrus pest vectored by ACP, caused ~ 80% decline in Florida citrus since 2005
- ACP detected in California in 2008
- HLB was detected in 2012
- >8,900 trees tested HLB positive and removed in California, no detections in commercial citrus
- ACP on untreated residential citrus trees are a significant risk

Photo by David Hall, USDA ARS

Photo by David Hall, USDA ARS

Area-Wide IPM

- "Total pest population management" leading to more sustainable pest control contrast with "field by field" control methods*
- Target highly mobile pests affecting crops across a landscape.
- Include all plant hosts within a defined geographic area, or an area isolated from other hosts.
- Use of multiple control measures to target key choke points in pest life cycle/phenology or most susceptible stages.

*FAUST, R. M., KOUL, O. & CUPERUS, G. 2008. General introduction to areawide pest management. Areawide Pest Management theory and implementation. Cambridge, MA, USA: CAB International, 1-14.

ACP in residential citrus

- Ongoing releases of *T. radiata* are made around citrus greening (HLB) quarantine zones by CDFA
- *T. radiata* has reduced ACP density, but risk of HLB in residential areas remain a threat to citrus production

CDFA Buffer Treatments

- Residential citrus within 250m of commercial orchards received pesticide treatments twice per year
- Treatments were effective in reducing ACP density
- Discontinued March 2023 due to CDFA budget constraints

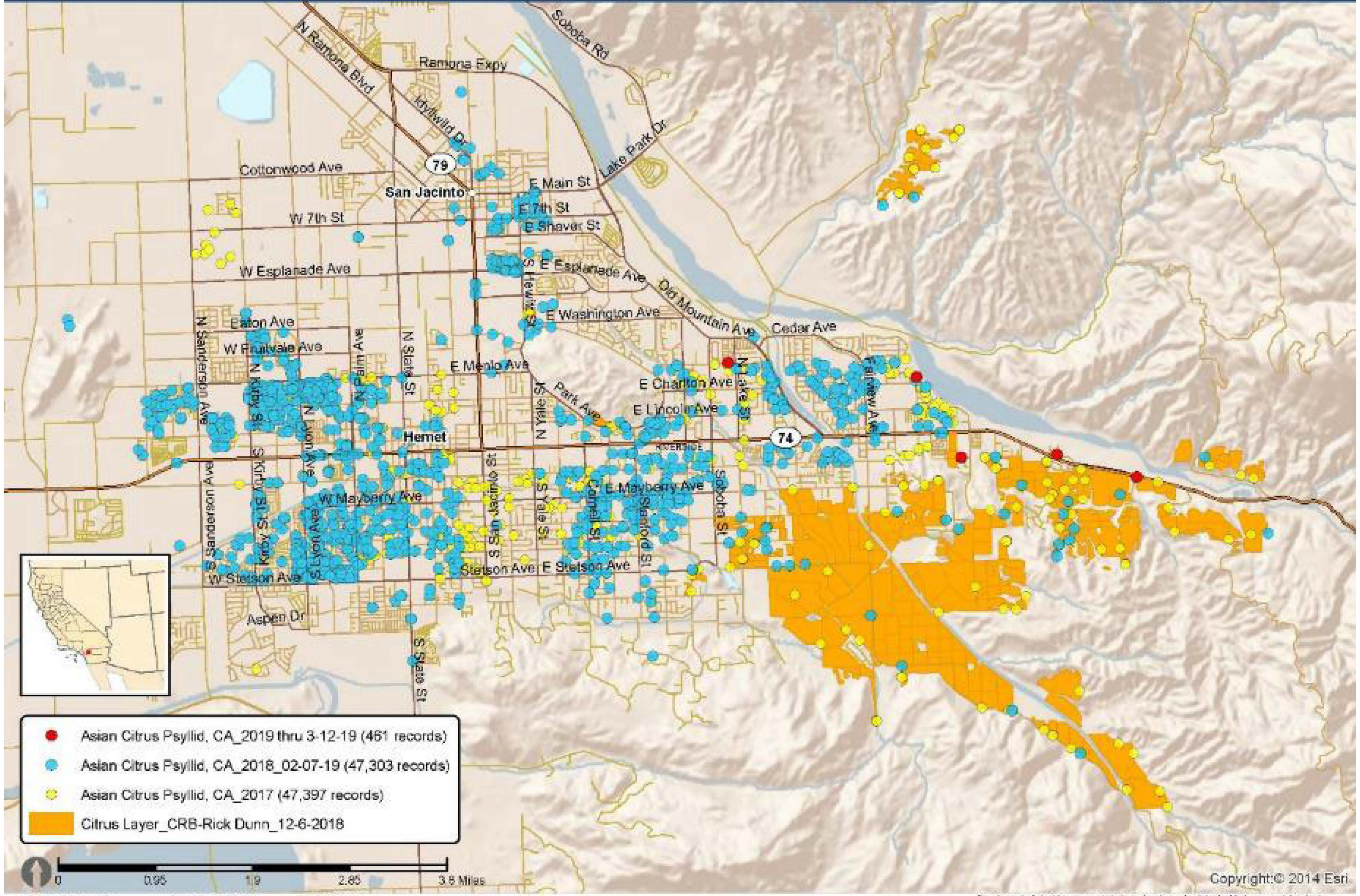
➢ Loss of chlorpyrifos for use to control sugar feeding ants leaves a large gap in growers IPM programs for citrus and grapes

➢ **Are there cost-effective options available to replace these treatments and ongoing *Tamarixia* release?**

USDA United States Department of Agriculture

CALIFORNIA, ARIZONA, NEVADA, BAJA CALIFORNIA, AND SONORA - 2019

Cooperative Program for Asian Citrus Psyllid (Diaphorina citri)



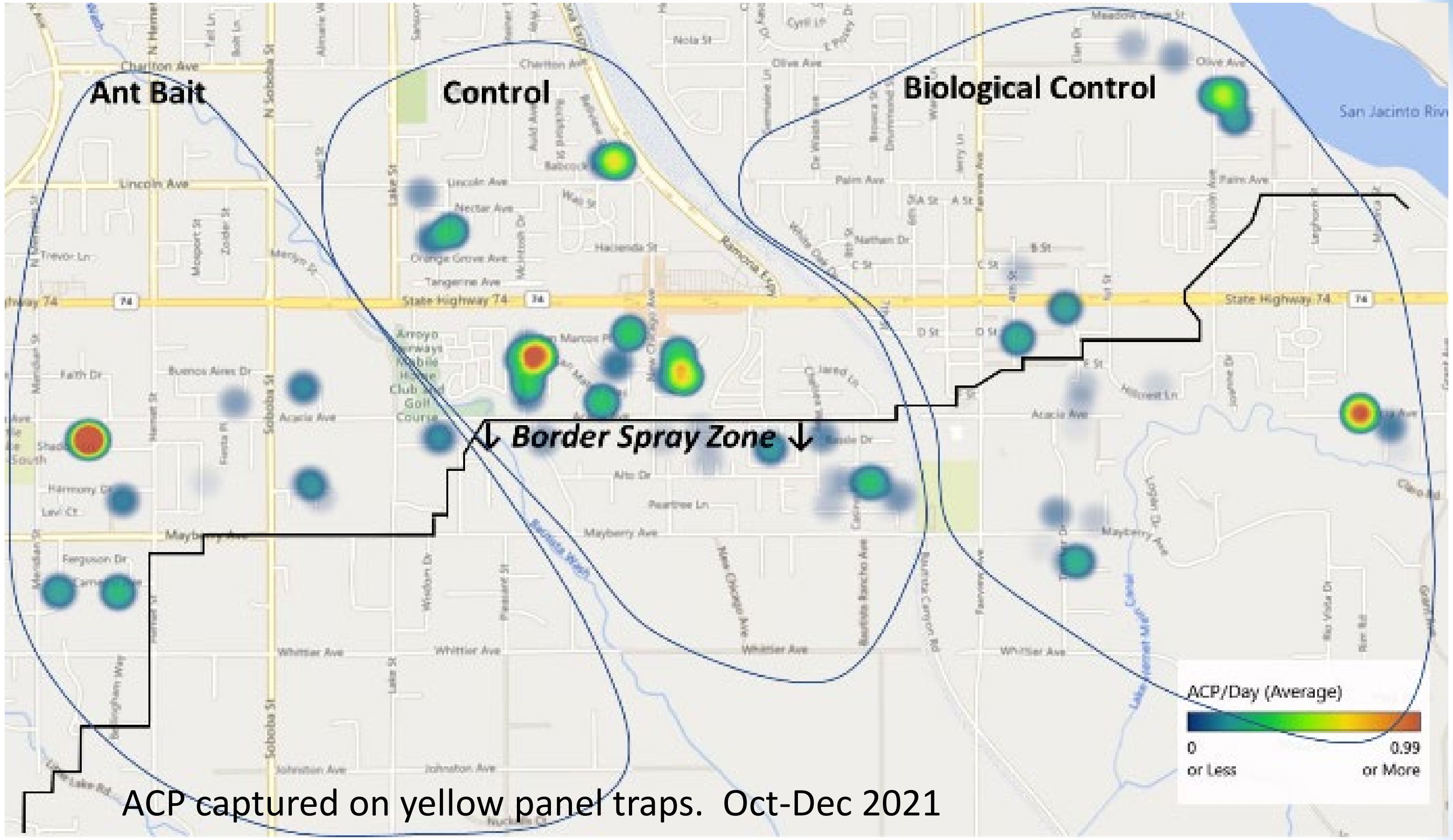
USDA, APHIS, PPQ
GIS Specialist
650 Capitol Mall, Suite 6-400
Sacramento, CA 95814

Coordinate System:
CA Teale Albers, NAD83
Date: 3/20/2019
Time: 12:34 hrs PT

Data Source:
CA Dept of Food & Agriculture
USDA, APHIS, PPQ
USDA, APHIS, IS

AW-IPM Study: 2019-2021

- Study conducted in the residential-commercial citrus buffer-zone
- Treatments:
    - Ant control – boric acid bait stations
    - Biocontrol – combined releases of *T. radiata* along 3 predator species
- Reductions in ACP populations as great as 75% relative to no-treatment control areas for both ant control and biocontrol areas



- 2022-2023 Single species releases of *C. comanche*, *D. pumilio*, *R. lophanthae* & *T. radiata*. Biweekly releases of 50 /tree

- A trend of lower ACP for some treatments but low ACP numbers for whole season made it difficult determine statistical significance

Why area-wide ant control?

- Sugar feeding ants known to interfere with biocontrol of many hemipteran plant pests including https://biocontrol.ucr.edu/asian_citrus_psyllid.html#ten
- When Argentine ants are removed or not present, biocontrol of ACP increases by 50 –to 99% (Kistner et al. 2016, Schall and Hoddle 2019, Milosavljević et al. 2021.)
- *Tamarixia* and native natural enemies are widely established across S. Calif. Ant control may be more effective than continuing to release natural enemies


(Photos: Mike Lewis, Center of Invasive Species Research, UCR).

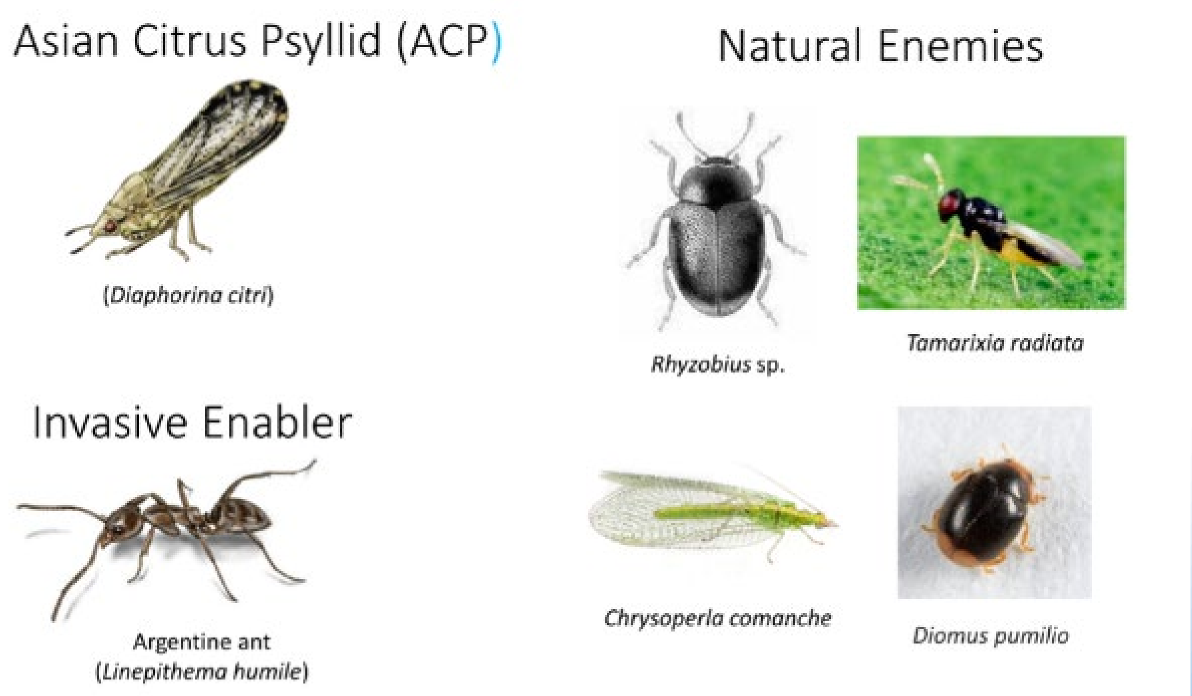
ACP biological control agents, augmentative release and classical BC

Tamarixa radiata, ACP parasitoid, family Eulophidae imported from Pakistan, 1st release in CA in 2011.

3 predator species:

- *Chrysoperla comanche,* green lacewing
- *Rhyzobius lophanthae,* scale eating ladybird beetle
- *Diomus pumilio,* psyllid and mealybug specialist coccinellid beetle
- *R. lophanthae & C. comanche* are available from commercial insectaries; *D. pumilio* is exclusively reared in colony by CRB
- All confirmed to feed on ACP in the field based on gut content analysis (Goldman 2017)
- Shown effective at reducing ACP populations in lab and field cage trials (Gómez-Marco et al. 2022)
- Other important predators: syrphids, minute pirate bugs

- Lower cost than *Tamarixia radiata*
  - *T. radiata* - $0.25-$0.78 per insect
  - *R. lophanthe*: $0.09 per insect
  - *C. comanche*: $0.04 per insect



- Goldmann, A., 2017. *Predators of Asian citrus psyllid (Diaphorina citri) in Southern California* (Doctoral dissertation, UC Riverside).
- Gómez-Marco, F., Gebiola, M., Simmons, G.S. and Stouthamer, R., 2022. Native, naturalized and commercial predators evaluated for use against *Diaphorina citri*. Crop Protection, 155, p.105907.

Help Us Save Our Citrus Trees

The Citrus Research Board and the Hemet Citrus Pest Control District is developing a toolbox of options to control a dangerous insect called the Asian citrus psyllid (ACP) – and **we need your help.**

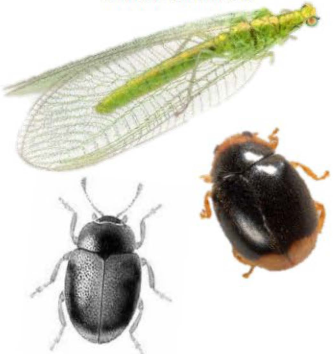
ACP spreads a devastating citrus pathogen that causes the plant disease Huanglongbing, or HLB. HLB kills all citrus trees it infects, and while it hasn't been found in Hemet yet, it is spreading in Los Angeles, Orange, and western parts of Riverside counties. As part of the area-wide effort to control the ACP, the Citrus Research Board is evaluating methods of controlling the Argentine ant in residential citrus trees. Argentine ant is an invasive ant that tends & protects ACP and other & garden pests from natural enemies while feeding on the sticky honeydew they produce.

You can help by:

Notifying the CRB or the pest control district of your interest in having beneficial insects released and/or allowing the evaluation of ant control methods in your property. This can not only reduce numbers of ants, but also provide control of citrus pests like ACP that Argentine ants protect. Three low-risk types of ant control will be tested: two low dose insecticide baits that foraging ants will take back to the colony causing the queen and the colony to die; and an ant trail-finding disruptant that stops ants from following their chemical trails to food sources in the landscape. Both of these methods are a very soft and targeted way to reduce the impacts of these invasive pests. Beneficial insects provide a natural way to control citrus pests like ACP as well as other garden pests, through a tactic known as biological control. These small, beneficial insects only feed on other insects and are not a nuisance to people or pets. Controlling the ants, will allow them to be more effective.

Argentine Ant

Beneficial Insects



Besides Helping to Protect Hemet's Citrus Trees, the Benefits Will Include:

- Observe science at work in your own garden.
- Reduce numbers of nuisance ants.
- Learn about the natural history of pests and the beneficial species that eat them.
- Contribute to the effort to limit the pest's spread and damage.

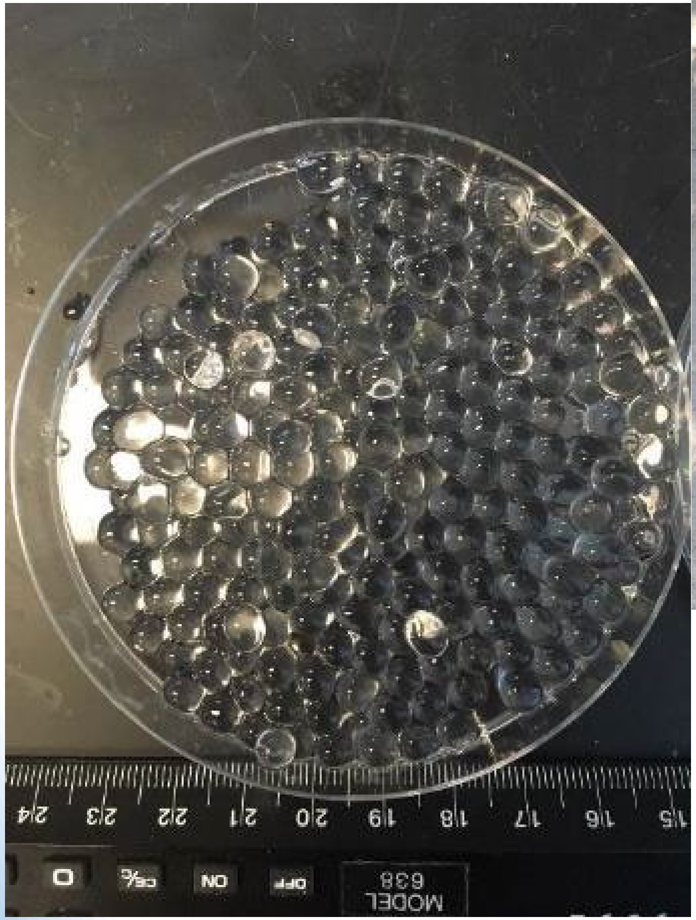
For more information:
Citrus Research Board
Dr. Raju Pandey
raju@citrusresearch.org
(951) 522-7666
Hemet Pest Control District
Bill Oesterlein
bill@rivcoag.org
(909) 725-6259

Are there alternatives to ongoing insecticide applications or biocontrol releases in residential areas?

Experimental Protocol

- Baits applied monthly to drip line of all trees on each property.
  - Hydrogel bait: Na alginate gel, 1 % boric acid & 25% sucrose & Z9-16:Ald as an attractant @ 10 gal/acre rate,
  - Antixx Plus: ant-bait labeled for residential use 0.07% Spinosad, 0.97% iron phosphate & sucrose @ 20 lbs. acre
- ISOTRAIL-AA (Shin Etsu), Argentine ant trail finding disruption (TFD) pheromone,
  - Z9-16:Ald, used at high rate of 1 m “rope” tied at ~ 1.5 on tree trunk, 400 mg Z9-16: Ald
- Applications from April-November 8 @ 30 d for baits (8), @60 d for TFD (4)
- Control (no treatment)
- 8-10 replicates per treatment over 2024-2025
- Biweekly monitoring for Argentine ants, ACP & BC agents
  - Sugar cotton balls
  - Tap samples
  - Traps

Added Hydrogel with boric acid for treatment on three large grove properties. With Dong-Hwan Choe, UCR



Argentine ant trail finding disruption pheromone

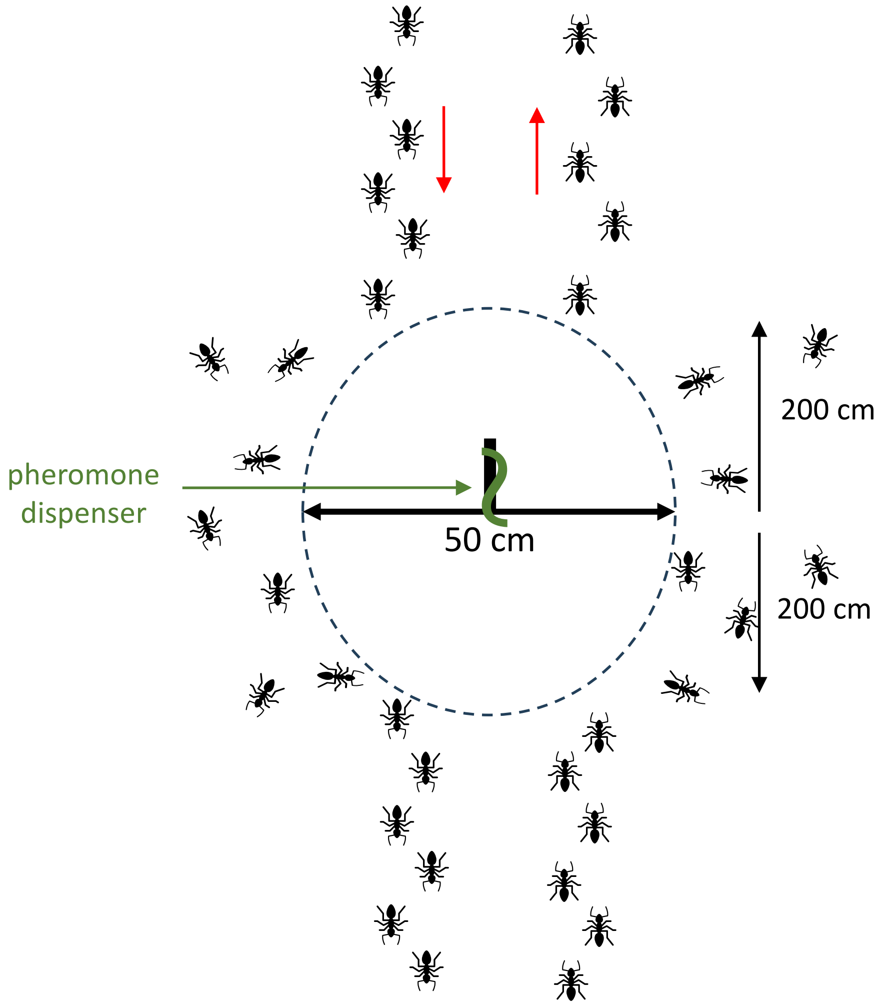
Undisrupted ant trail

Disrupted ant trail

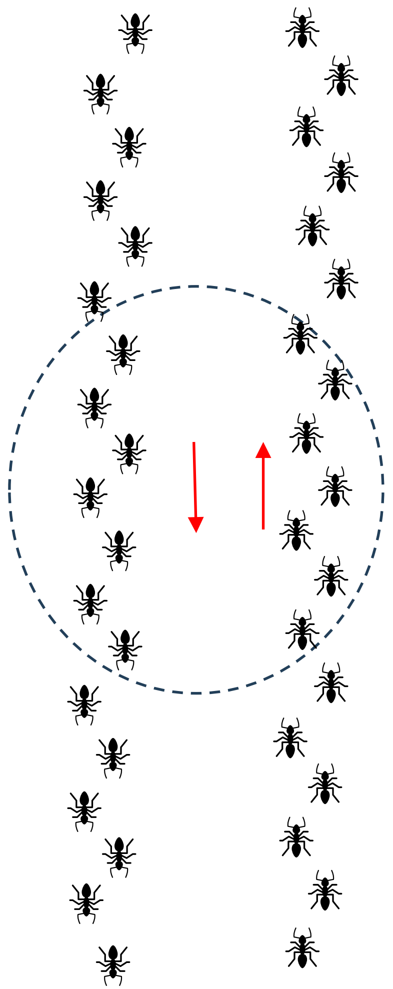
Ants use irrigation pipes as "superhighways"*, placement of trail finding disruption twist-ties may work to stop ants from tending ACP in citrus trees & increase biological control of ACP. * UCR, M. Hoddle Laboratory Research

Ant TFD dispenser placement, 20 cm hollow tube dispensers, on irrigation pipe, & stake or around tree trunk (1 m)

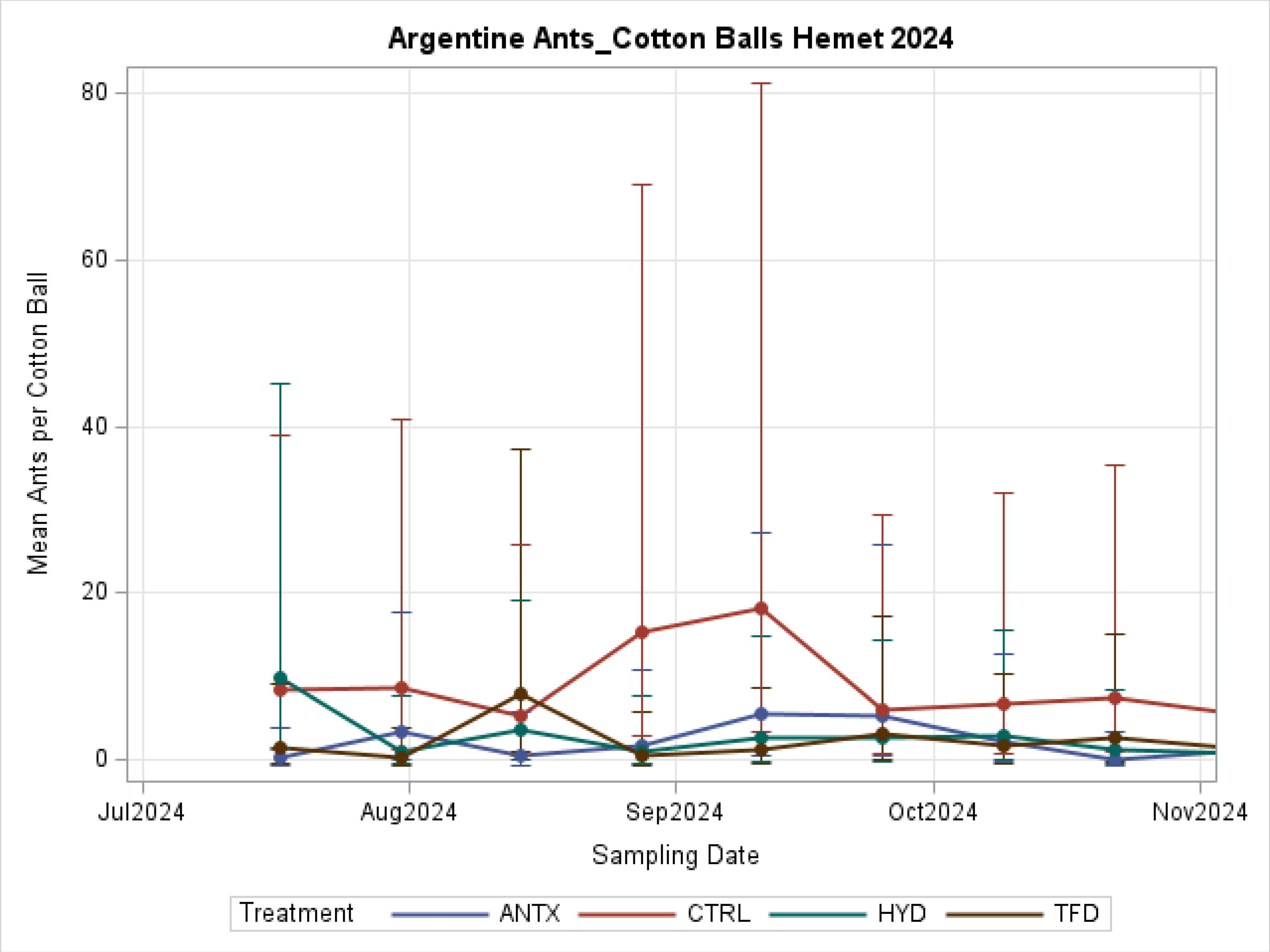
Argentine Ant Trail “Disrupted”



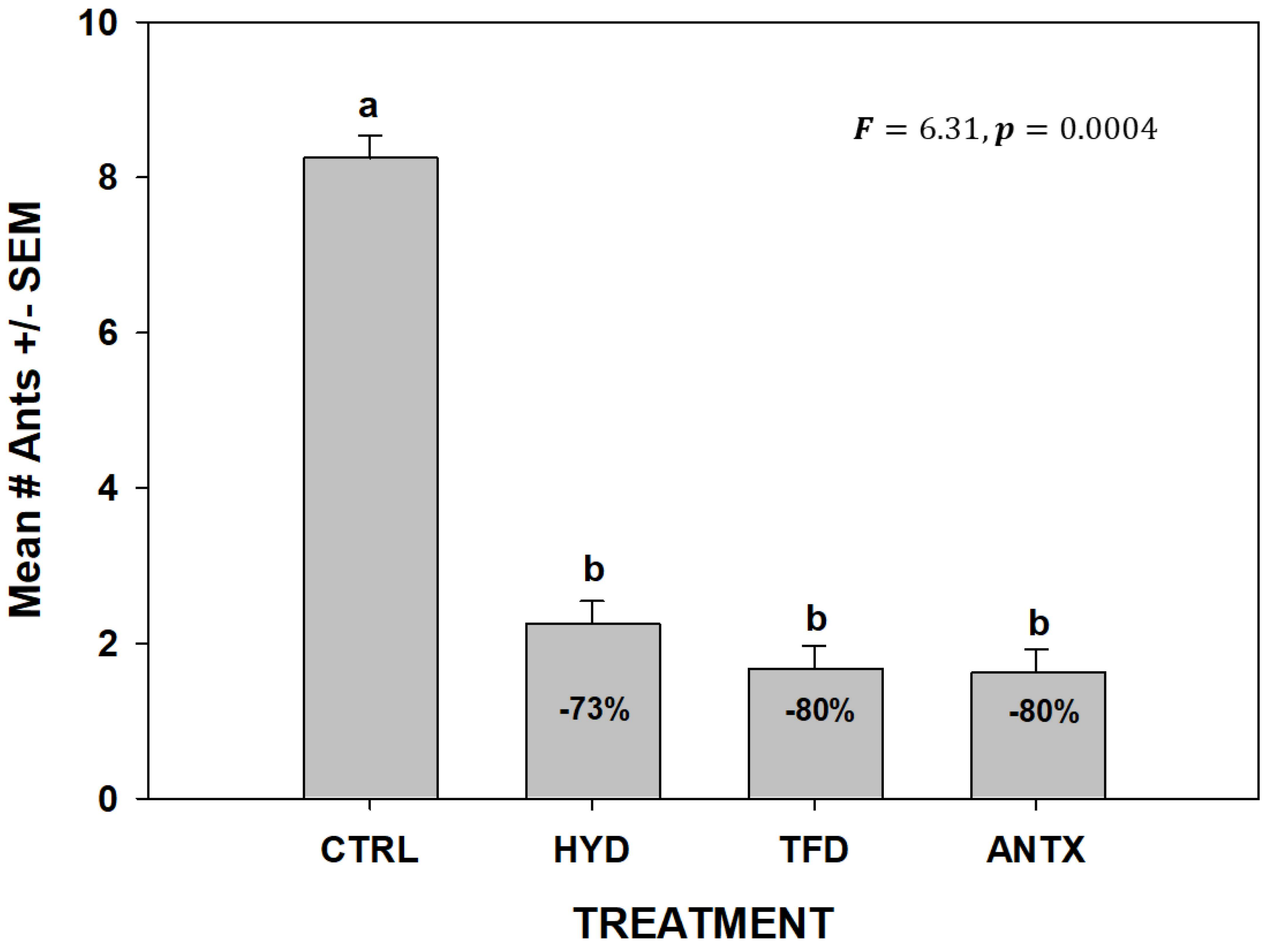
Argentine Ant Trail “Undisrupted”

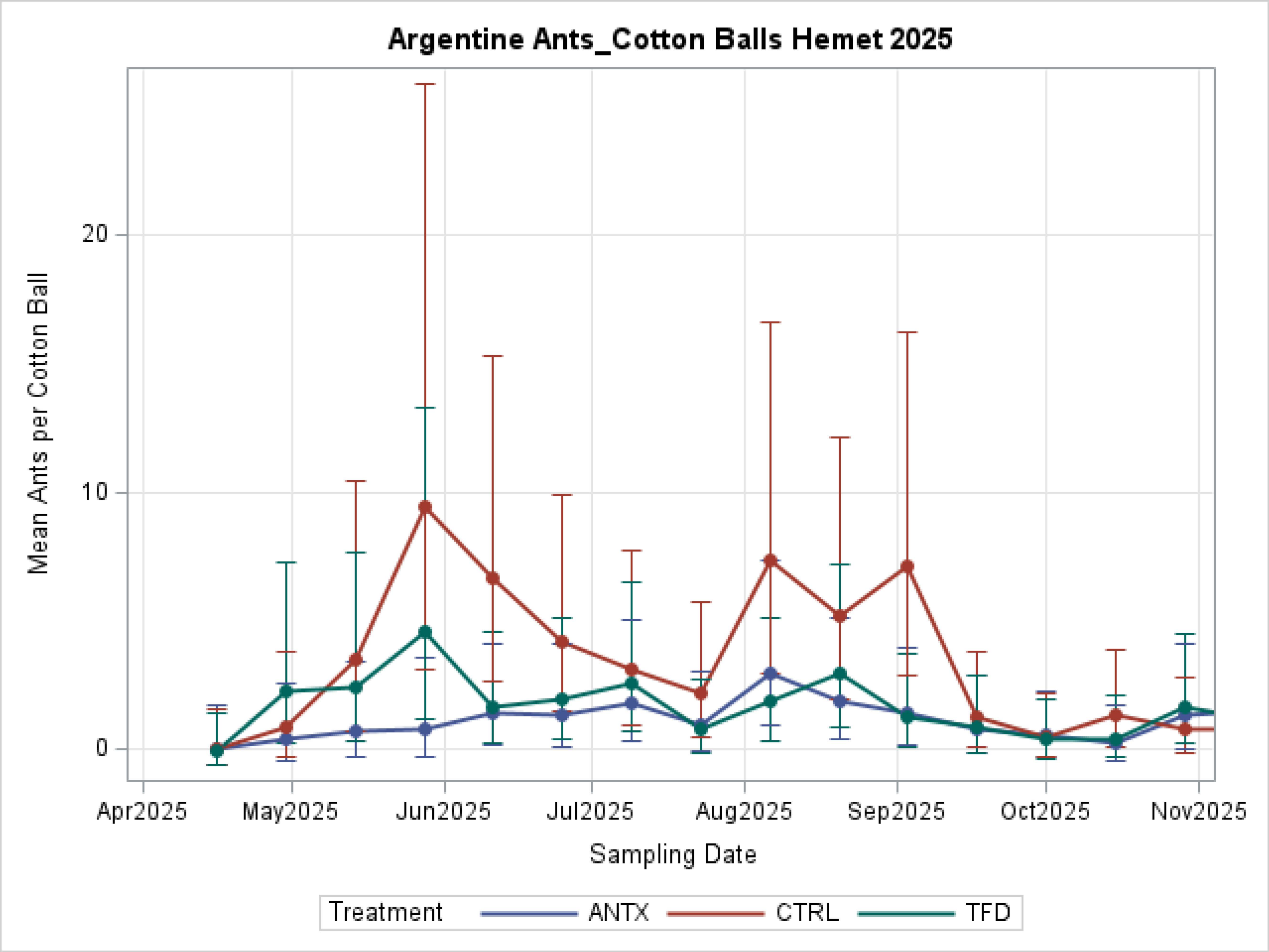


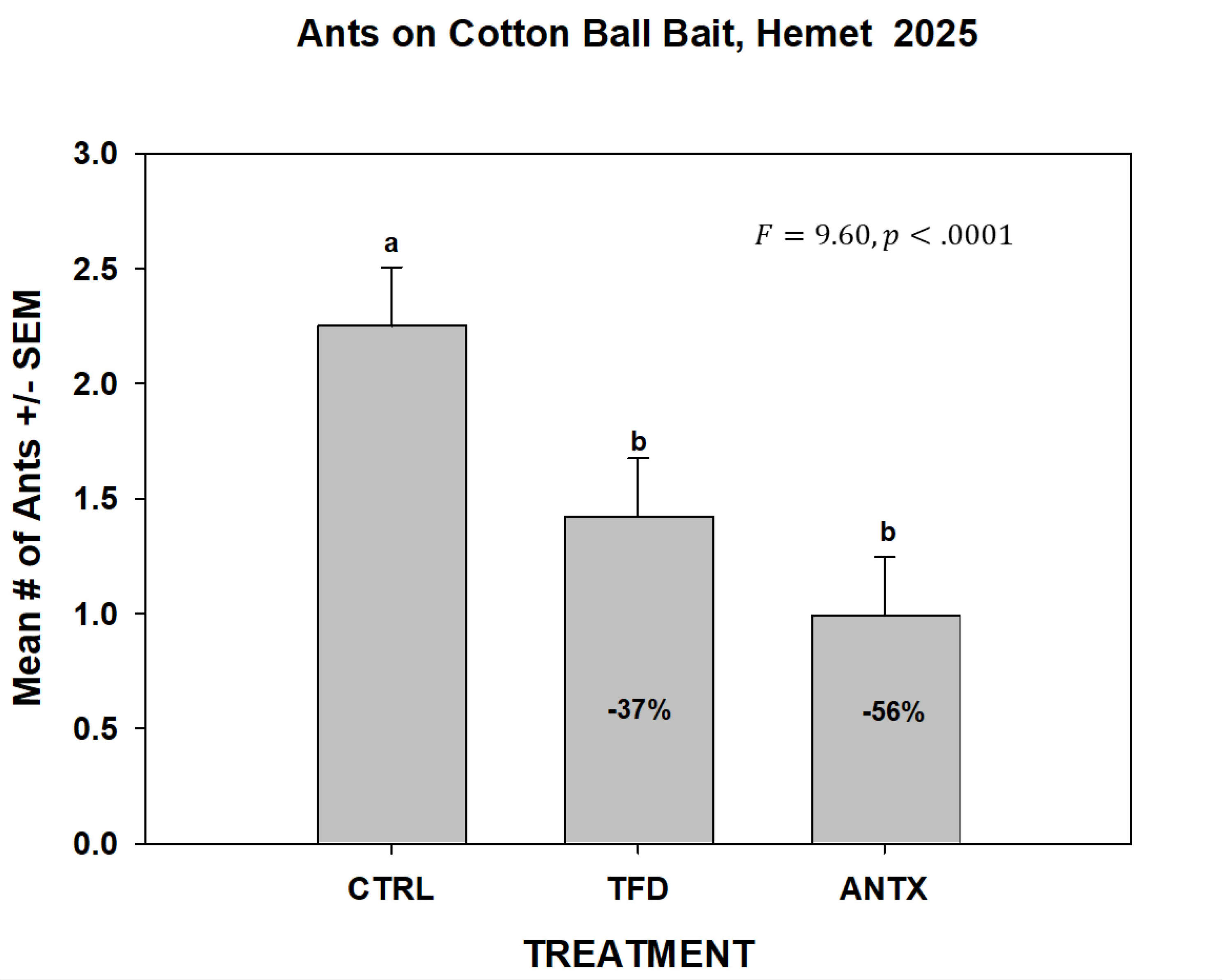
Tanaka, Y. et al 2009. Trail-following disruption in the invasive Argentine ant with a synthetic pheromone component (Z)-9-hexadecenal. Sociobiology 54:139–152


Argentine Ants_Cotton Balls Hemet 2024
Mean Ants per Cotton Ball
0
20
40
60
80
Jul2024
Aug2024
Sep2024
Oct2024
Nov2024
Sampling Date
Treatment
ANTX
CTRL
HYD
TFD

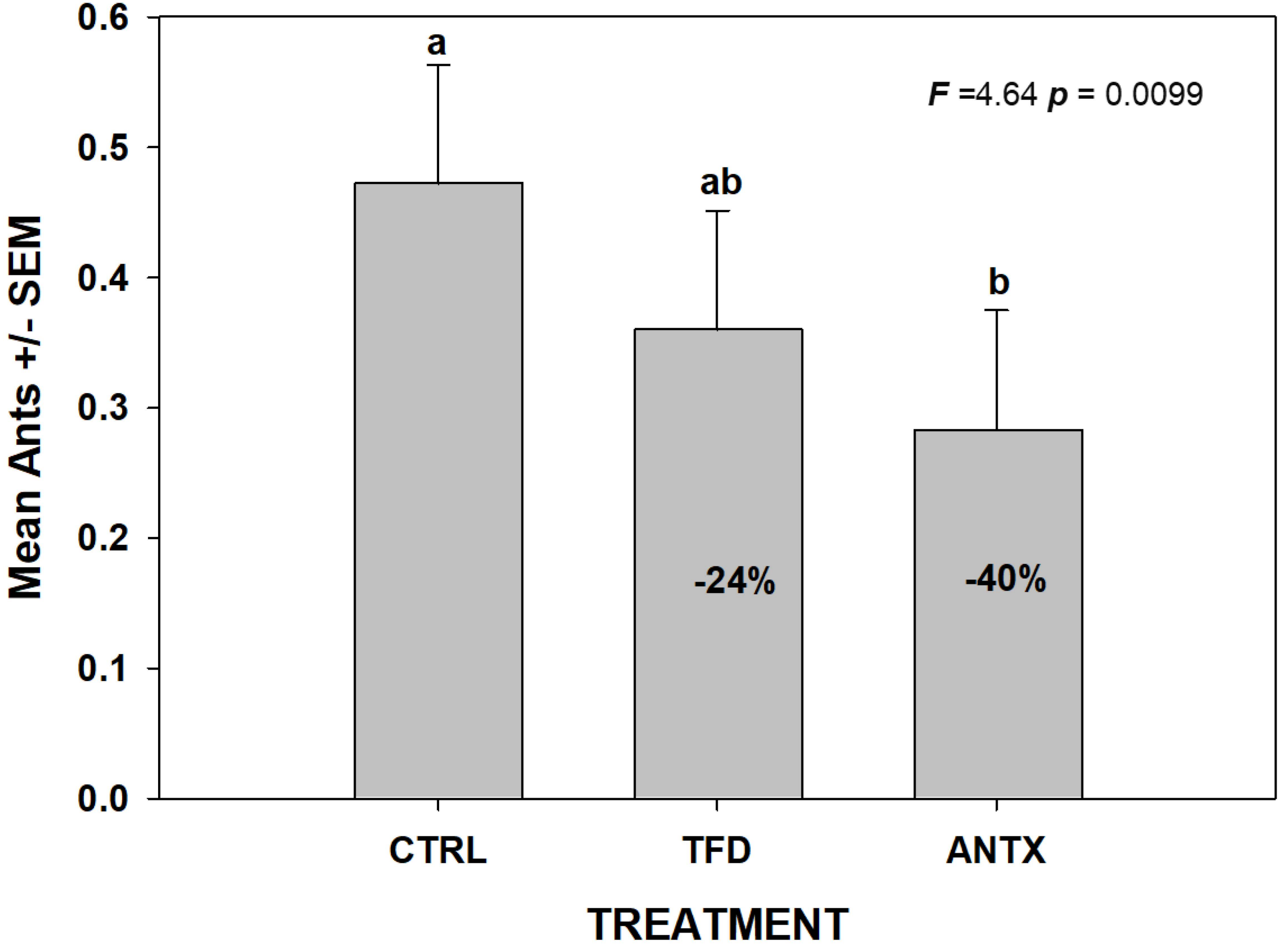
Ants on Cotton Ball Bait, Hemet 2024

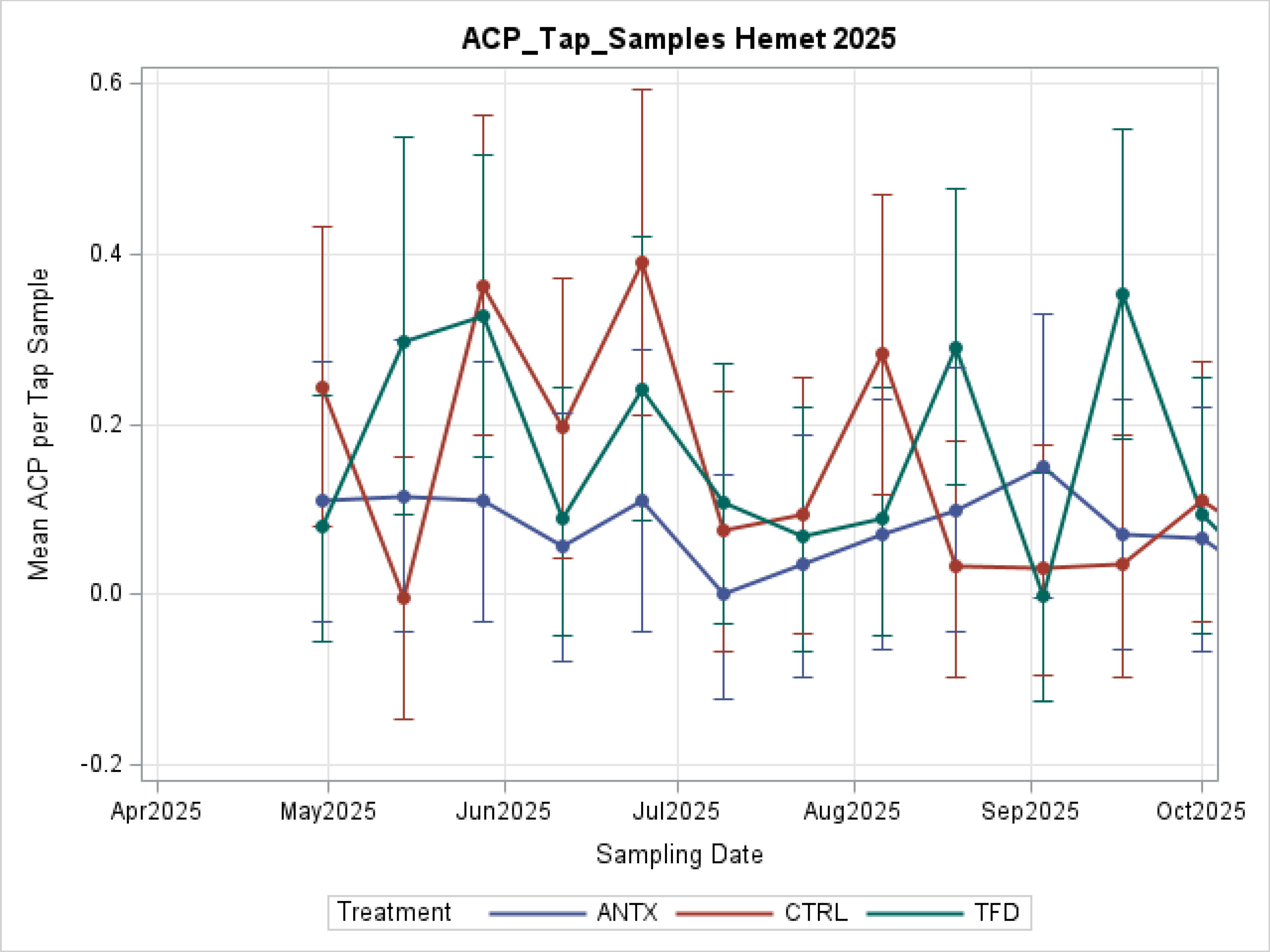


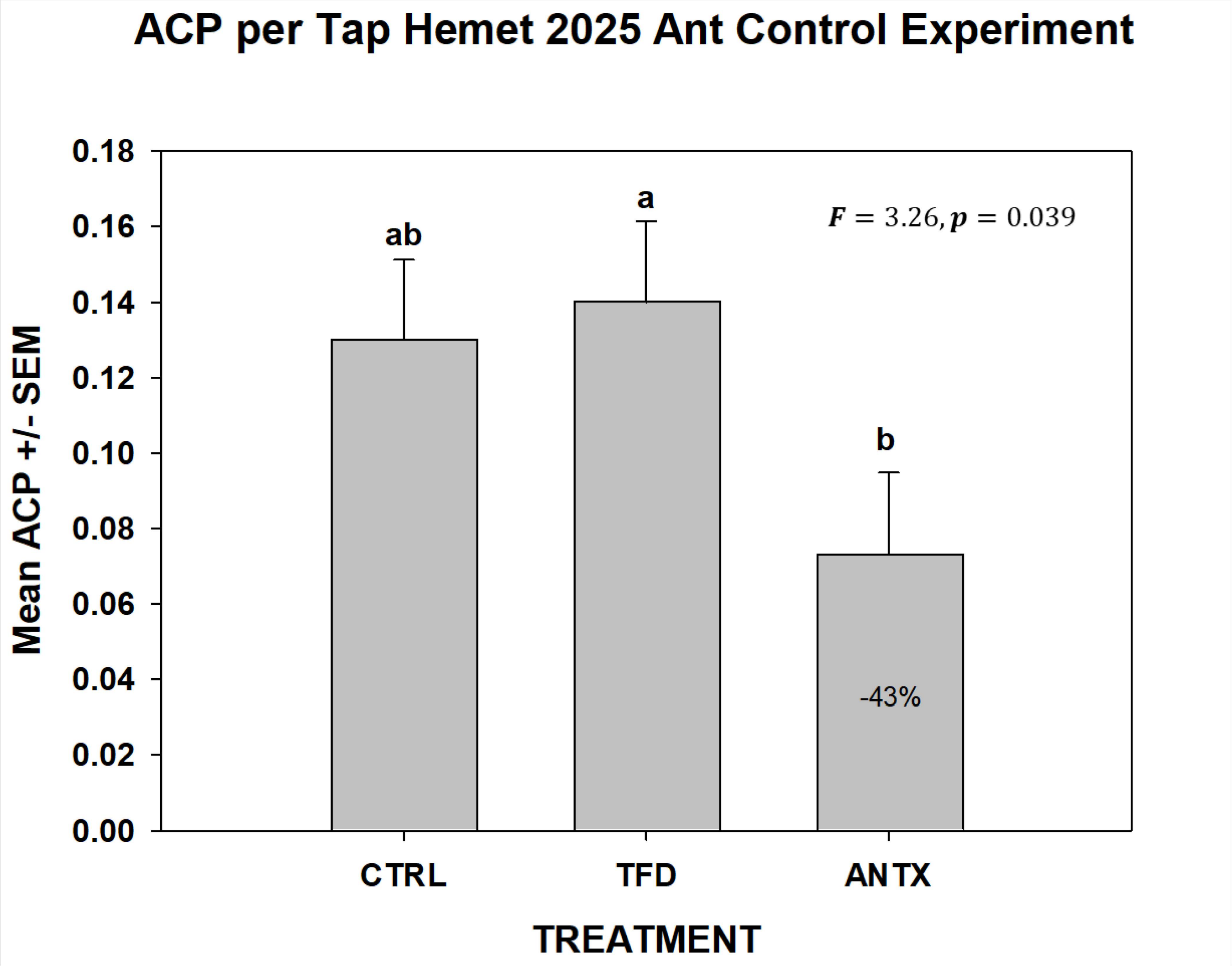

Argentine Ants_Cotton Balls Hemet 2025
Mean Ants per Cotton Ball
0
10
20
Apr2025
May2025
Jun2025
Jul2025
Aug2025
Sep2025
Oct2025
Nov2025
Sampling Date
Treatment
ANTX
CTRL
TFD


Ants on Cotton Ball Bait, Hemet 2025
Mean # of Ants +/- SEM
3.0
2.5
2.0
1.5
1.0
0.5
0.0
$F = 9.60, p < .0001$
a
b
b
-37%
-56%
CTRL
TFD
ANTX
TREATMENT

Ant Tap Samples Hemet 2025




ACP_Tap_Samples Hemet 2025
Mean ACP per Tap Sample
0.6
0.4
0.2
0.0
-0.2
Apr2025
May2025
Jun2025
Jul2025
Aug2025
Sep2025
Oct2025
Sampling Date
Treatment
ANTX
CTRL
TFD


ACP per Tap Hemet 2025 Ant Control Experiment
Mean ACP +/- SEM
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
ab
a
b
$F = 3.26, p = 0.039$
-43%
CTRL
TFD
ANTX
TREATMENT

Summary (1)

- Significant reductions in AA populations door yard citrus over two years
- Low ACP populations make it hard to show an effect of ant control
- Ant control will affect ACP populations thru action of established & native enemies
  - ➢What ant control rates are needed to have impact in area-wide control programs?
- Antixx Plus is a registered product and could be an effective treatment for area-wide control of ACP in residential citrus
  - ➢Cost of ant control vs. ongoing biocontrol releases?
- Hydrogel bait is currently unregistered but some options for growers to use are being developed.

Summary (2)

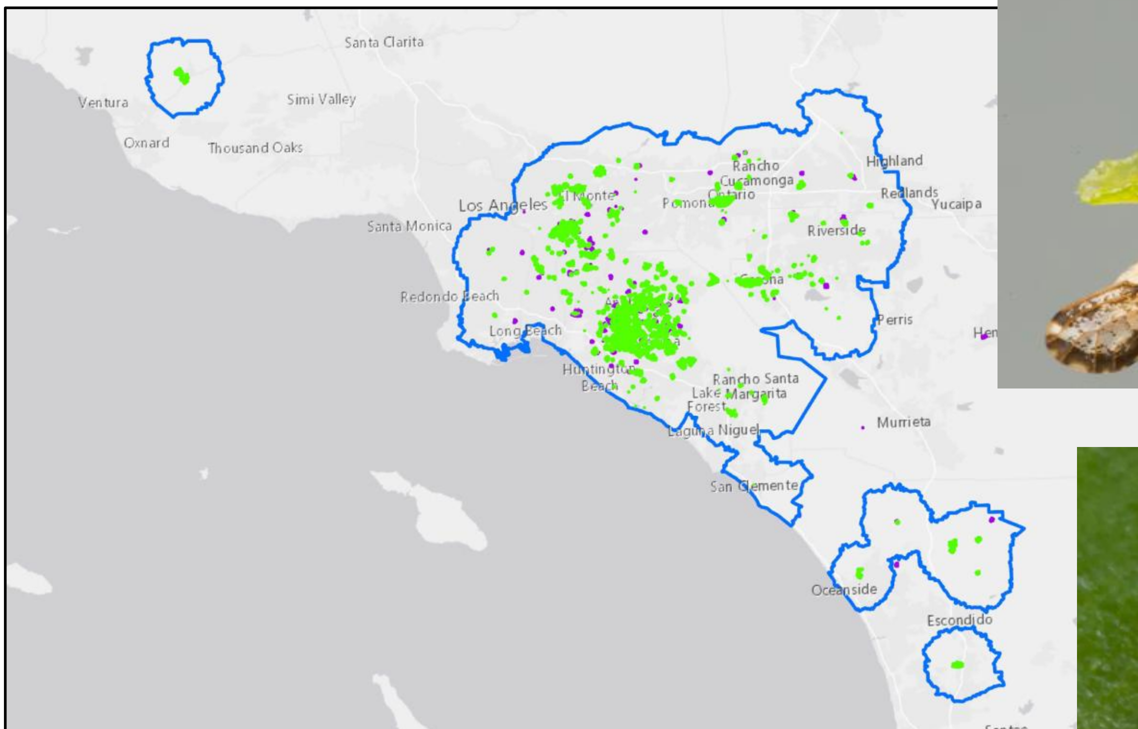
- ISOTRAIL AA is not (yet?) a commercial product
  - ➢We used a high rate of 1 m ropes on the tree trunk which reduced ant capture on cotton balls
  - ➢Placement of dispensers on irrigation pipes did not work
  - ➢More work needed to determine application rates & best dispenser placement
  - ➢We need to think carefully about sampling strategy, probably need to place cotton balls in canopy

What are the prospects for incorporation of area-wide ant control into the ACP residential control program?

Acknowledgements

- Ivan Priego, Molly DeWitt, Carlos Pimienta, James Boyd (CRB/CASS)
- Six-legs Interns (UCR)
  - David Jacinto
  - Ofelia Doroteo
  - Mayra Cuevas
- Timo Rohula, Bodil Cass, Dong-Hwan Choe, Matt Daugherty, & Kerry Mauck (UCR)
- Eugenia Garcia & Tom Greene (USDA)
- Enrico Ferro (P.C.A., Area Wide ACP liaison for SC)
- Grace Radabaugh (CDFA)
- Bill Osterlein (Hemet Citrus Pest Control District)
- Chris Hedstrom & Peter McGhee (Pacific Biocontrol)
- Shin-Etsu

Effectiveness of ACP management in HLB quarantine zones



photos: Mike Lewis

Matt Daugherty, UC Riverside, mattd@ucr.edu

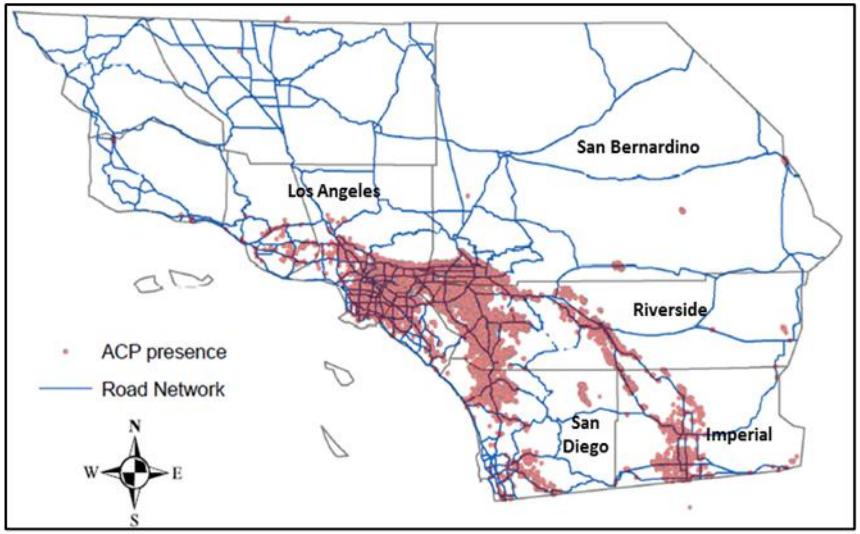
Current status of ACP, HLB in California

ACP is widespread in Southern California

- present, but rare, in Northern California

> 10,000 HLB cases detected

- 4 distinct quarantine areas
- concentrated in residential citrus



1. Residential ACP treatments

Following *C*Las/HLB detection, tree treated, then removed

Inspection of neighboring properties in 250 m radius, treatment of ACP hosts

- combination of foliar pyrethroid + systemic neonicotinoid

2. Classical biological control of ACP

Tamarixia radiata

- nymphal ectoparastoid
- preference for 4^{th}, 5^{th} instars
- prone to host-feeding

Imported from Pakistan

- host-range testing at UCR

First releases late 2011

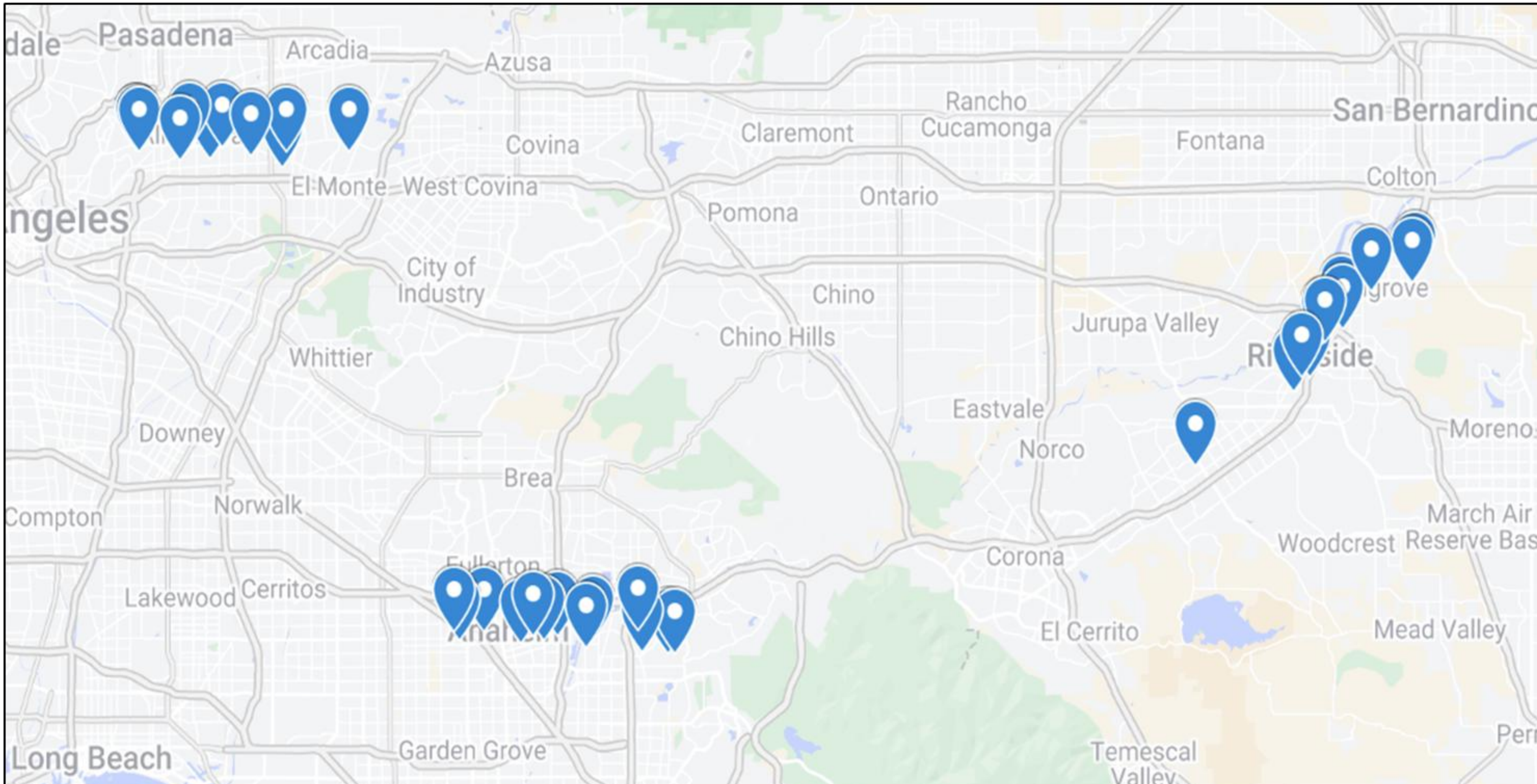
> 30 M released to date by CDFA

How effective is ACP management in HLB quarantine zones?

1. Is there evidence that treatments are having an impact?

2. Are treatments and biocontrol compatible?

Study design

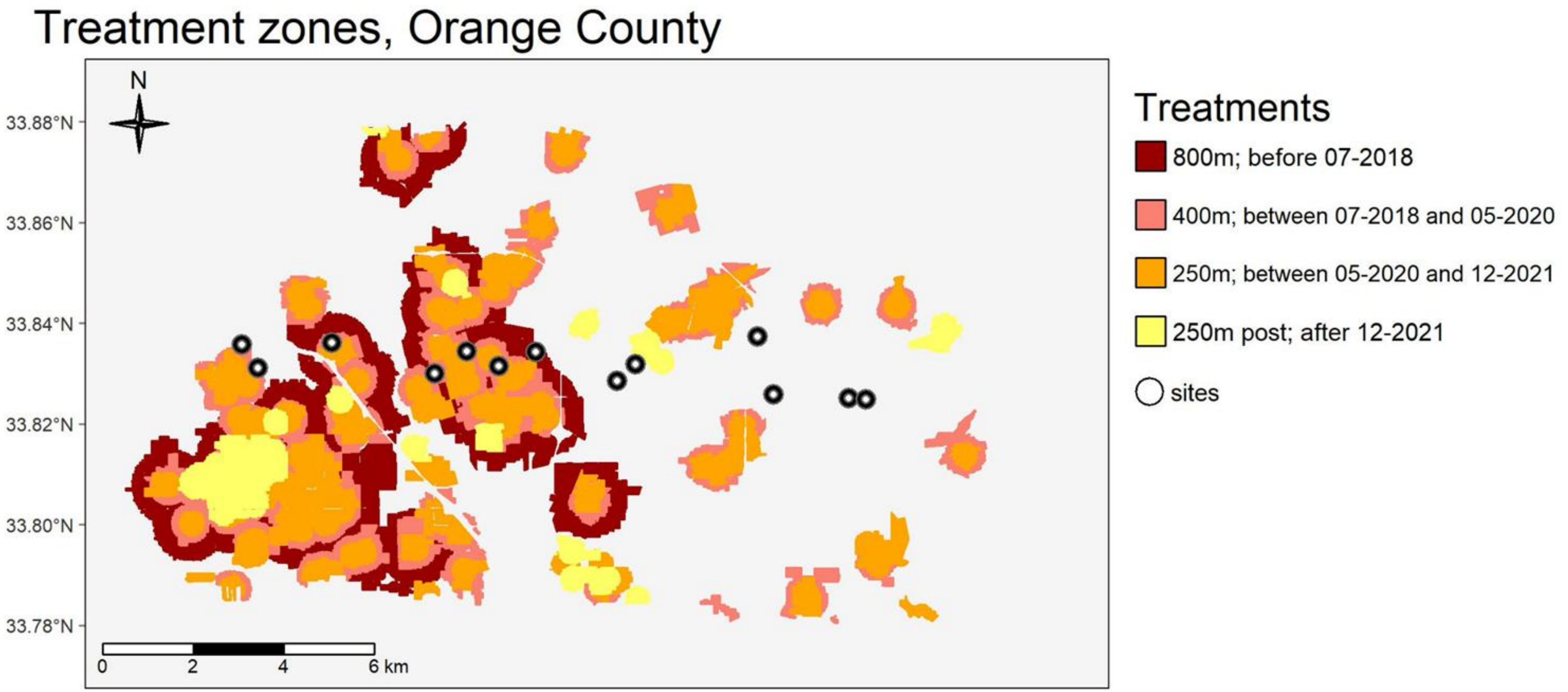


- repeated surveys at 35 sites in and around HLB treatment zones

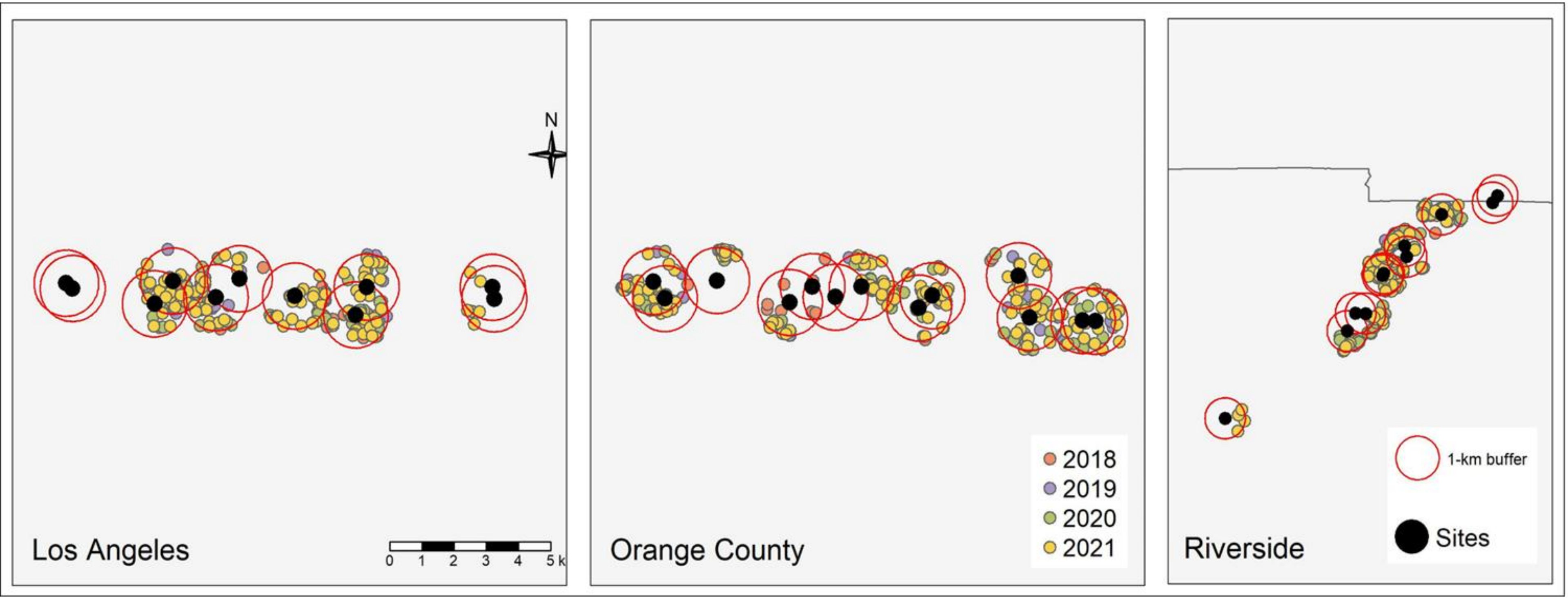
5+ years of censuses, 2x per month (> 7500 observations)

- flush availability
- timed counts of ACP adults in tree canopy
- collected/inspected flush (ACP adults, eggs, nymphs, mummies, parasitoid emergence)
- sites within and outside of HLB treatment zones
- estimates of *Tamarixia* releases in surrounding area

Fit with zero-inflated negative binomial or binomial GLMMs

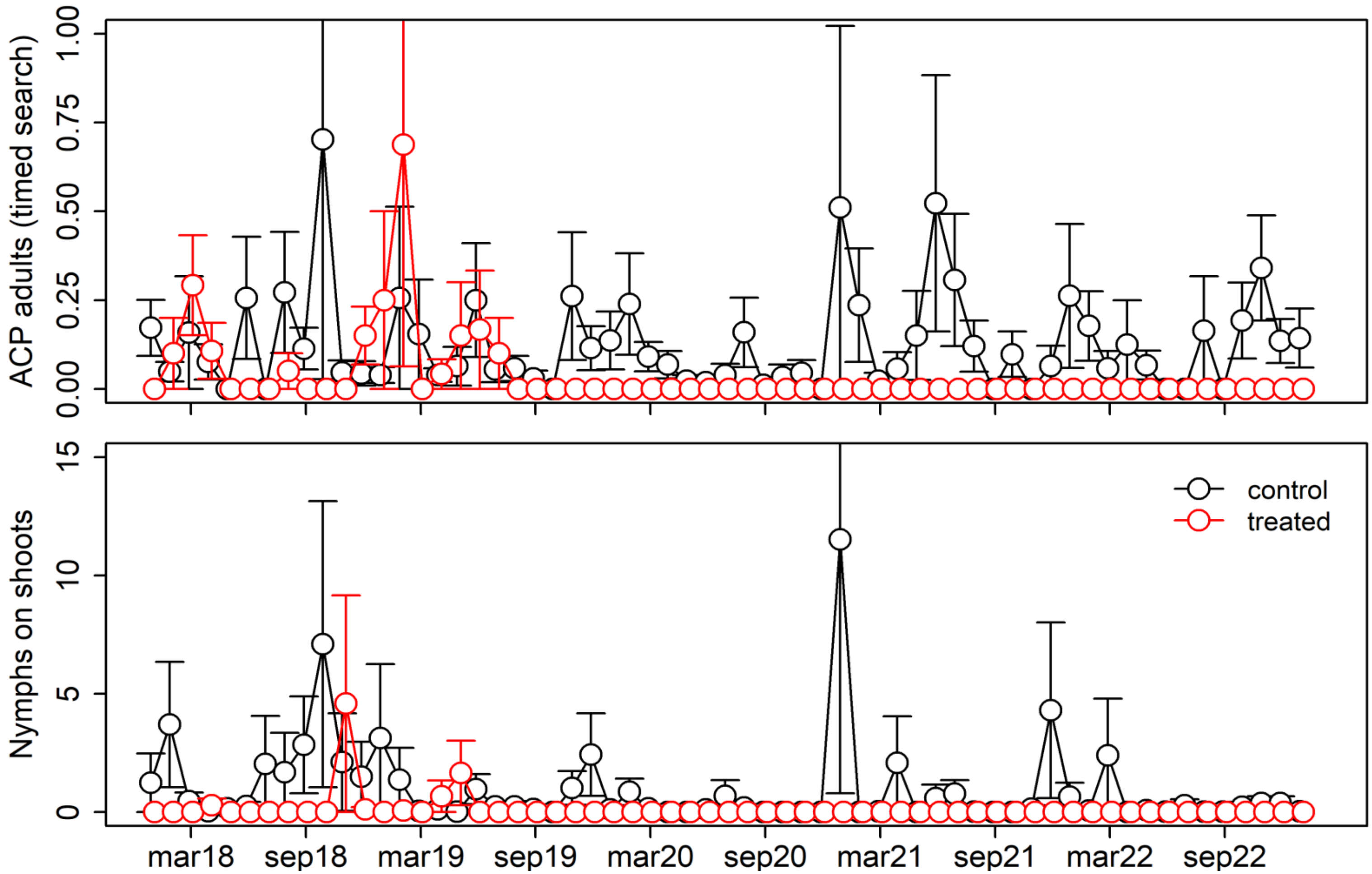


- sites within and outside of HLB treatment zones



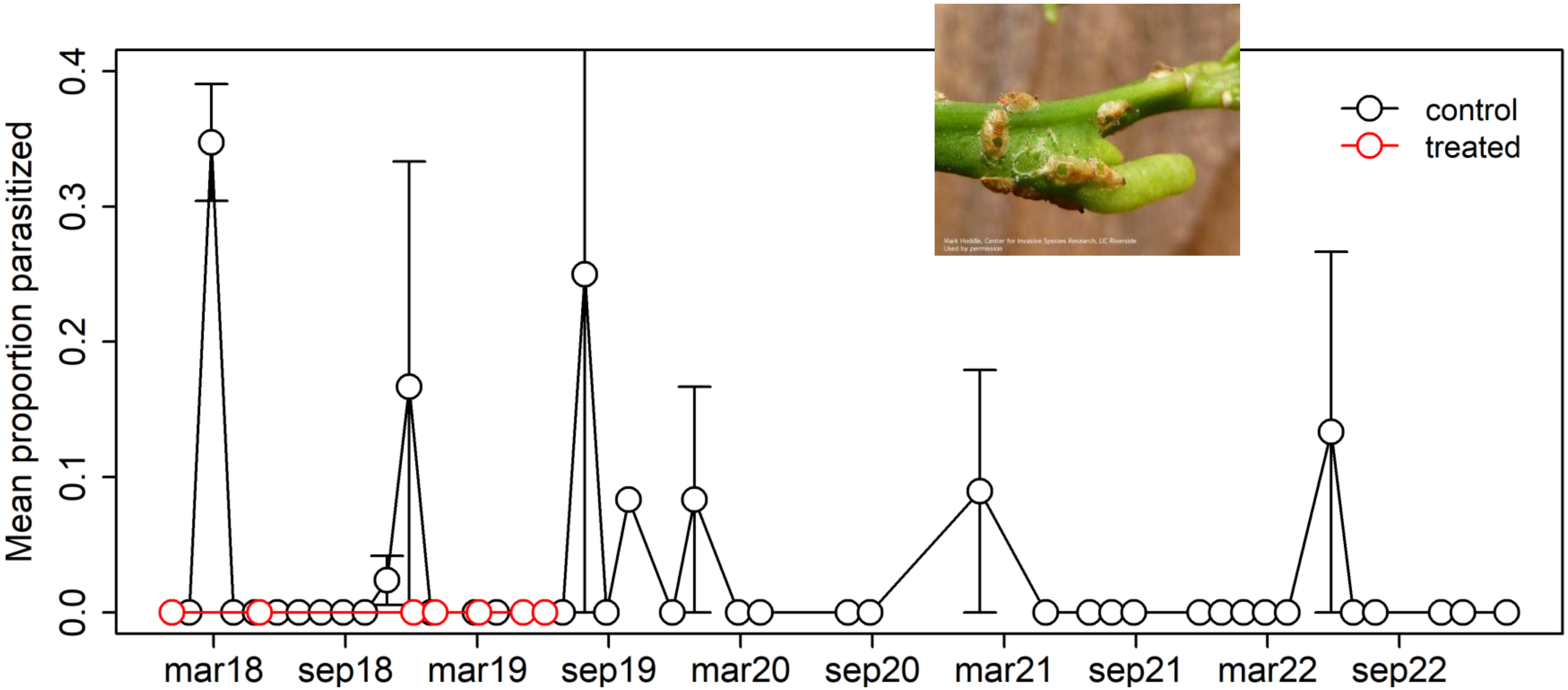
- estimates of *Tamarixia* releases in surrounding area

ACP – significant treatment-by-time interaction



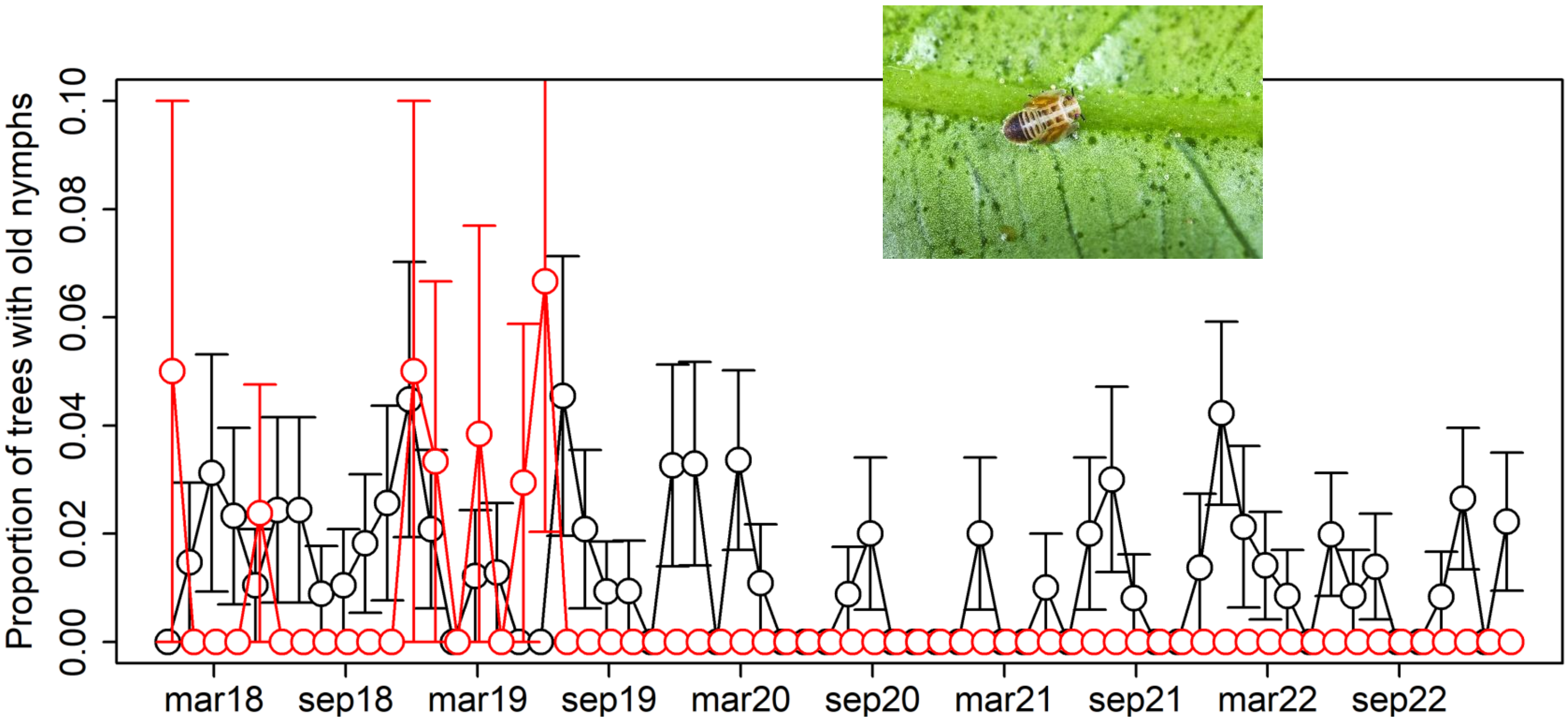
- ACP rare in treated sites after 2019

Parasitism ~13% overall, as high as 50%



- modest parasitism overall, in part due to a lack of suitable host stages for parasitism

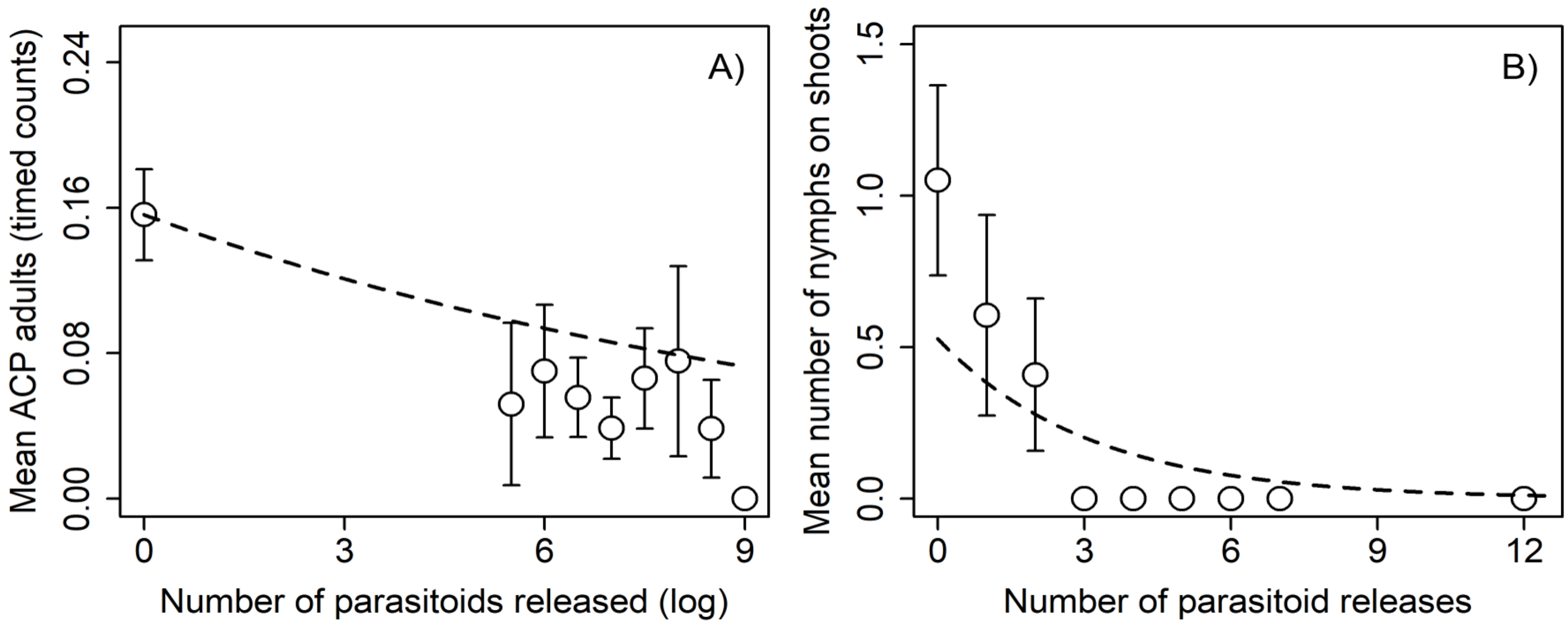
$4^{th}/5^{th}$ instar nymphs absent in treated sites after 2019



- treatments indirectly limit parasitism by limiting available hosts (but maybe non-target impacts on parasitoids too)

ACP – effects of *Tamarixia* releases

Even in the absence of much parasitism, effects of *Tamarixia* are apparent



- larger releases or more releases reduce ACP densities
- evidence of host feeding?

Effectiveness of ACP management in quarantine?

Insecticide treatments strongly reduce ACP densities

- cumulative, persistent declines in treated areas

Conservative estimate of the impact of biocontrol

- need more separation between treatments and releases

Larger, more frequent *T. radiata* releases reduce ACP

- substantial host-feeding by *Tamarixia* justifies continued augmentative releases?

Effectiveness of ACP management in quarantine?

Hard to determine (with available data) if vector control in quarantine areas slows pathogen/disease spread

- ACP densities have declined in some parts of Southern California

- but, elements of pathosystem may undermine effective disease management

David Morgan

Rachael Massie

David Phong

Hector Zumbado-Ulate

Greg Simmons

Can the Sterile Insect Technique provide an alternative to pesticides for controlling the spread of a major pest of citrus, the Asian Citrus Psyllid, in California?

California Department of Pesticide Regulation Grant # 23-PMG-R001

Jedeliza B. Ferrater[1], Gregory S. Simmons[2], Matthew P. Daughtery[1], & Paul F. Rugman-Jones[1]*

[1]University of California, Riverside, California; [2]USDA-APHIS-PPQ, Salinas, California

Photo: Mike Lewis, UCR

Background

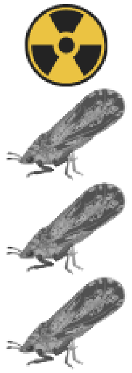
- HLB is the most devastating citrus disease worldwide.
- Florida has lost >80% of its citrus production since 2010.
- California now accounts for ~79% of U.S. production ($2.55 billion).
- Infected trees have been found in Southern California.
- HLB is associated with the pathogen *Candidatus* Liberibacter asiaticus (*C*Las)
  - ACP is the major vector of *C*Las
  - ACP transmits *C*Las into the phloem when it is feeding on citrus flush.
  - The bacterium multiplies in the tree's phloem tissue, blocking the flow of nutrients
  - And killing the tree within 3 to 5 years.
- Best management practices center around removing infected trees, and **<u>controlling ACP</u>**.

Birth control for bugs: how does the sterile insect technique (SIT) work?

1

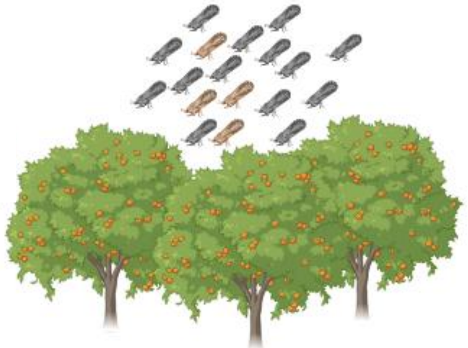
Insect Rearing: “Clean” psyllids are bred in large numbers in specialized insectaries on curry leaf, *Bergera koenigii*, a citrus relative that cannot harbor the bacterium that causes citrus greening.

2



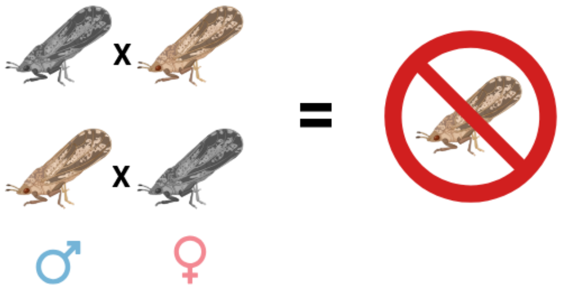
Sterilization: Adult psyllids are sterilized using X-ray radiation. This does not affect their ability to fly or mate, but does make them incapable of producing offspring.

3



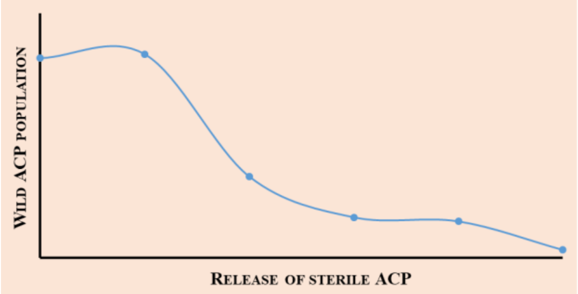
Release: The sterile psyllids are released into a grove where they mingle with the wild population. Releasing clean adults is a key component of the process since, as adults, the sterile psyllids are no longer able to effectively acquire and transmit citrus greening.

4



Mating: Sterile psyllids mate with wild individuals and although these encounters typically result in the production of eggs, the eggs are not fertile. Thus, no new psyllids are created.

5



Population Decline: Over time (i.e. multiple releases), sterile individuals continue to outcompete fertile ones, fewer psyllids are born, and the overall population of the Asian citrus psyllid drops.

From: “Using the ACP as a weapon against itself”
Citrograph, Vol. 16, No. 2

Wait! Won't you be releasing thousands of vectors, albeit sterile ones?

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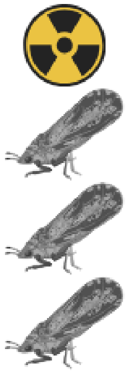

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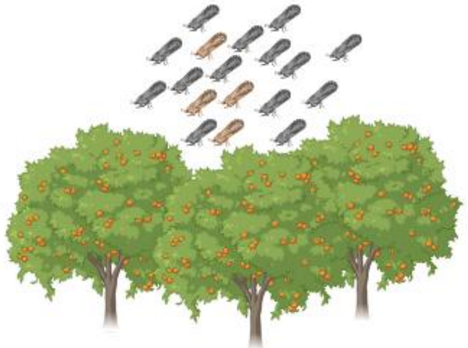

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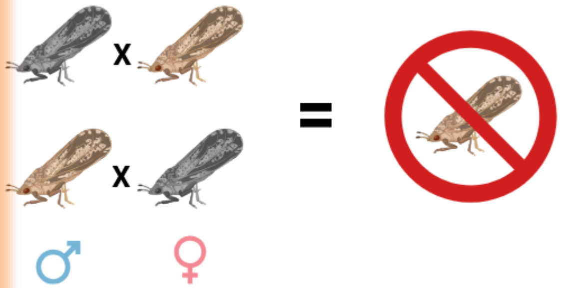

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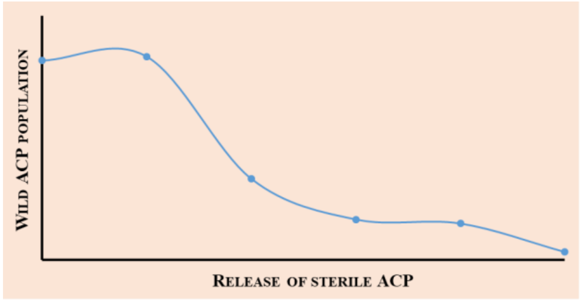

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ducing offspring.

Release: T

and transm

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Phytopathology • 2018 • 108:1089-1094 • https://doi.org/10.1094/PHYTO-01-18-0012-R

Ecology and Epidemiology

e-Xtra*

The Asian Citrus Psyllid Host *Murraya koenigii* Is Immune to Citrus Huanglongbing Pathogen '*Candidatus* Liberibacter asiaticus'

Vitor H. Beloti,† Gustavo R. Alves, Helvécio D. Coletta-Filho, and Pedro T. Yamamoto

First, second, and fourth authors: Department of Entomology and Acarology, 'Luiz de Queiroz' College of Agriculture, University of São Paulo, C.P. 9, Piracicaba, SP, 13.418-900, Brazil; and third author: Centro de Citricultura Sylvio Moreira, IAC, C.P. 4, Cordeirópolis, SP, 13490-970, Brazil.

Accepted for publication 10 April 2018.

Arthropods in Relation to Plant Disease

Transmission Parameters for *Candidatus* Liberibacter asiaticus by Asian Citrus Psyllid (Hemiptera: Psyllidae)

K. S. Pelz-Stelinski,[1,2] R. H. Brlansky,[3] T. A. Ebert,[1] and M. E. Rogers[1]

J. Econ. Entomol. 103(5): 1531–1541 (2010); DOI: 10.1603/EC10123

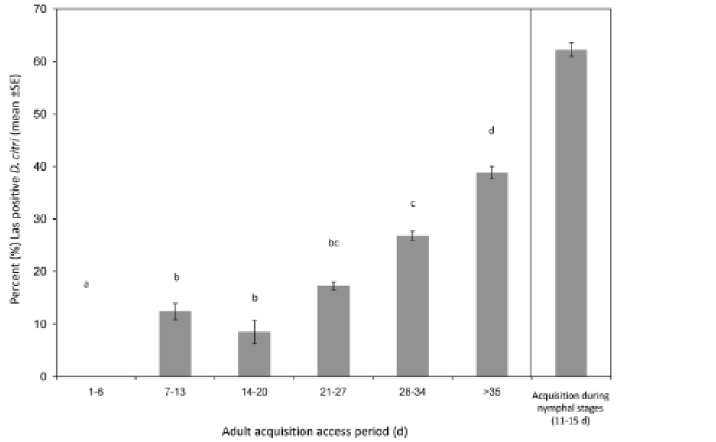


Fig. 1. Percentage of *D. citri* (mean ± SE) that tested positive for Las in laboratory experiments after feeding on infected citrus for AAPs lasting 1–45 d (adults) or for the duration of nymphal development from the egg stage through the fifth instar (11–15 d). Presence of Las was assessed using qPCR after each AAP. AAPs for adults were grouped by week for analysis. Means for adult AAPs with different letters are significantly different (α = 0.05; Fisher protected LSD).

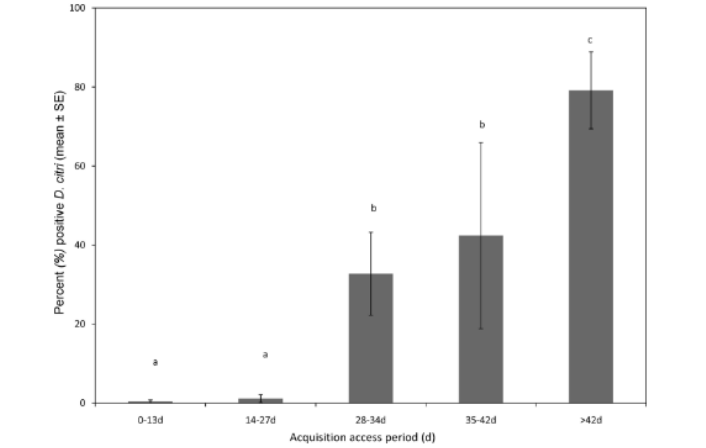
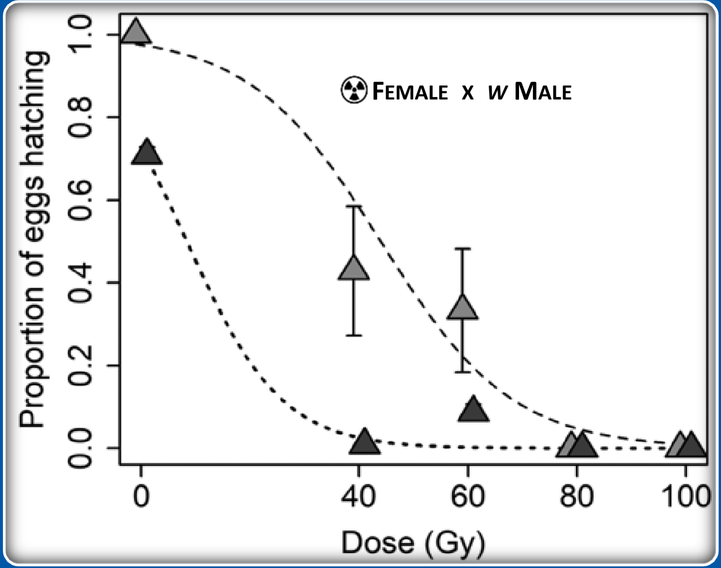
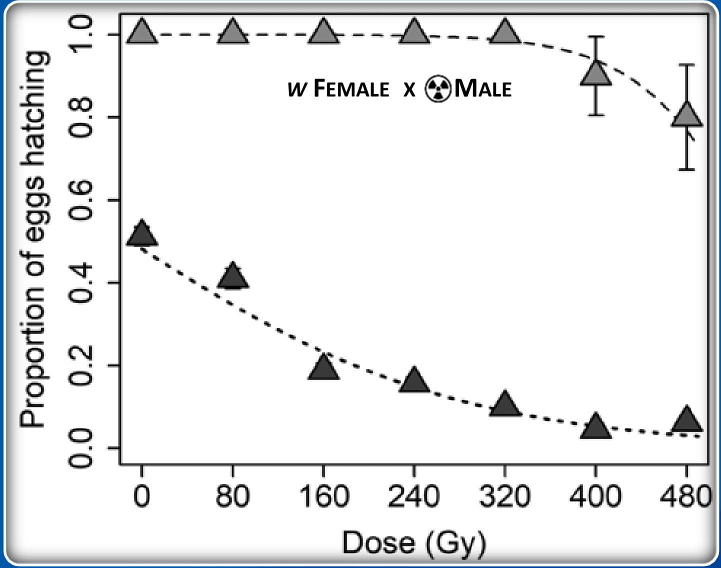
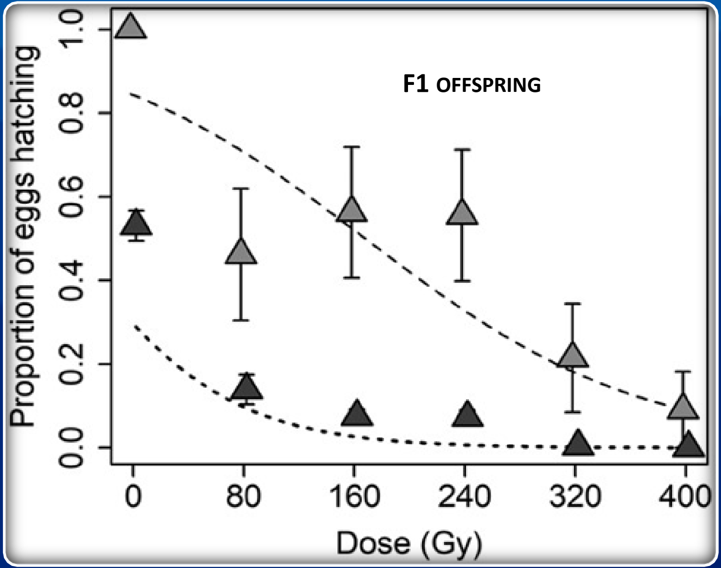


Fig. 2. Percentage of *D. citri* (mean ± SE) that tested positive for Las under field conditions after feeding on infected citrus for AAPs of 1–51 d. Presence of Las was assessed using qPCR after each AAP. AAPs for adults were grouped for analysis. Means for adult AAPs with different letters are significantly different (α = 0.05; Fisher protected LSD).

Earlier work: effective radiation dose





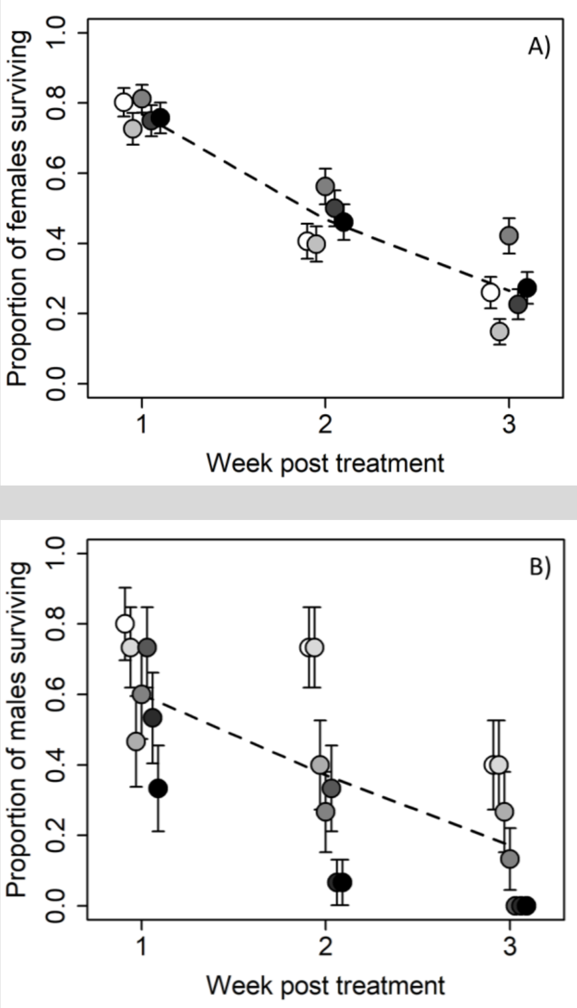


- Lighter gray shows the proportion (±SE) of females for which at least one egg hatched
- Darker gray shows the proportion (±SE) of all eggs that hatched.

- Females were more sensitive than males
- 320Gy X-ray radiation of male ACP results in 100% sterility of the F1 offspring (inherited sterility)
- ~70% of irradiated ACP males died within 2 weeks

Ferrater et al. (2024), https://doi.org/10.1093/jee/toae098

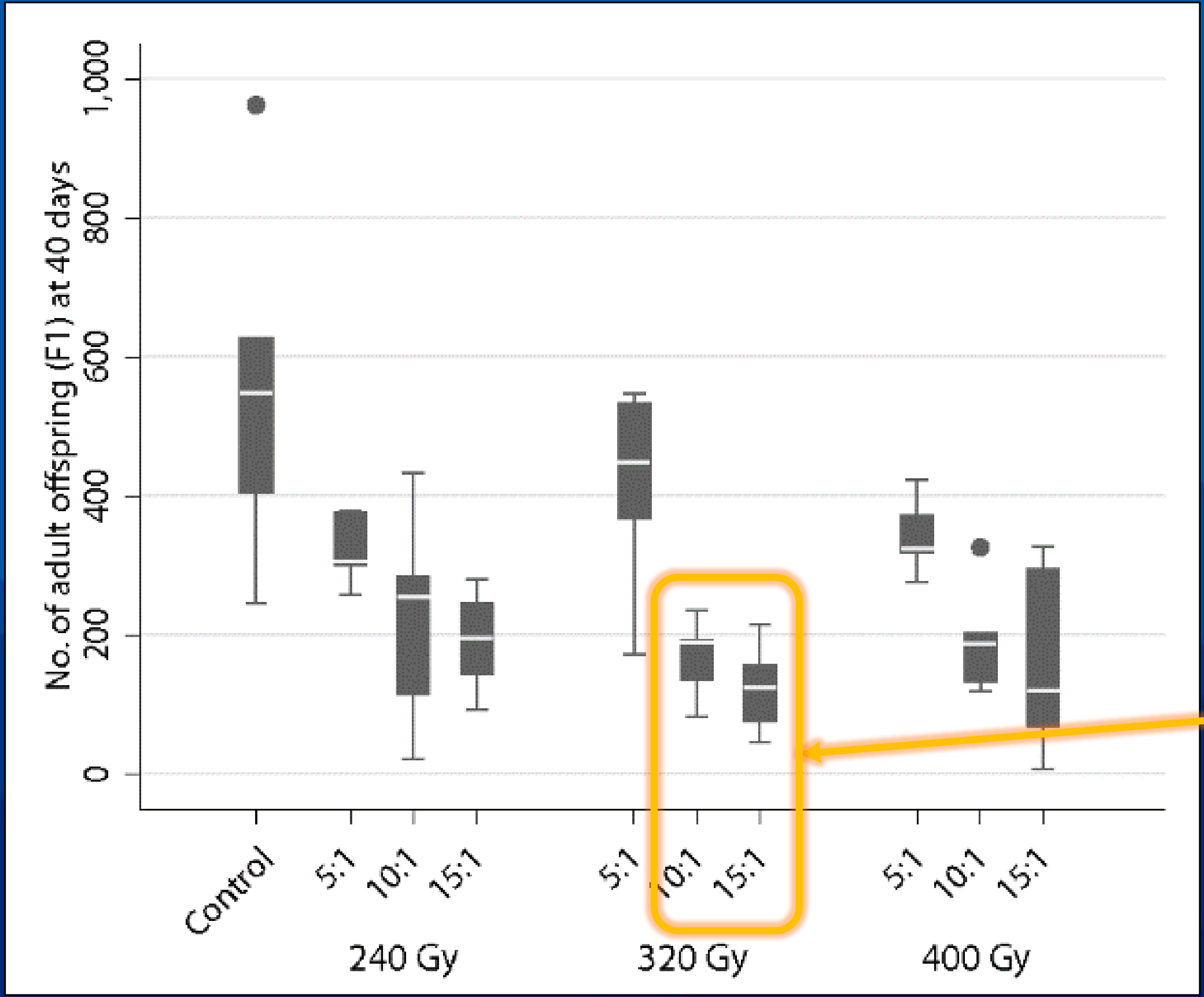
… & survival



- Darker colors indicate increasing dose

USDA-APHIS CHRP Agreement #AP21QS&T00C148

Earlier work: overflooding ratio [OFR]



Effective release rate
(overflooding ratio*)

At 320 Gy, ratios of 10:1 and 15:1 suppressed population growth up to 78%

(Ferrater *et al.* in prep.)

Looks good, but can it work in the field?

23-PMG-R001

Photo: Mike Lewis, UCR

Preparation of sterile ACP?

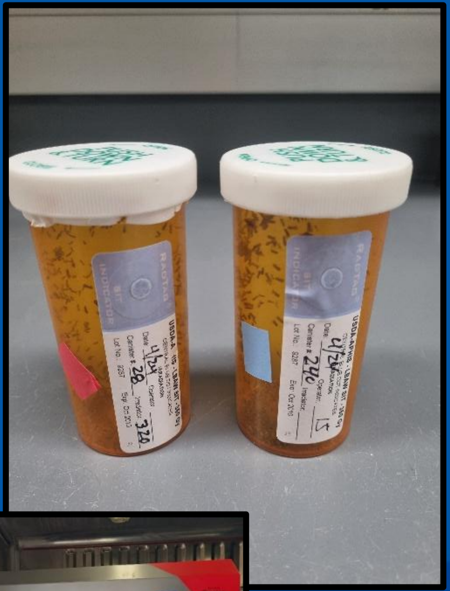
Mass rearing of psyllids

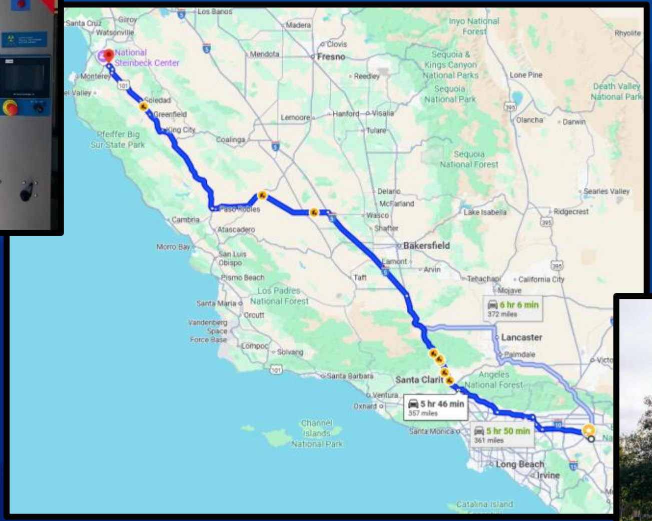
- *C*Las^{-ve} Adult ACP reared in UCR I&Q
- On potted curryleaf plants (*Bergera koenigii*) – not a reservoir for HLB!
- Insectary room ~ 6’ x 10’, >14 cages
- 200 ACP added to each cage
- At 80^{o}F, yields ~3,000 ACP per cage after 25 days
- >40,000 adult ACP in one month




Preparation of sterile ACP?

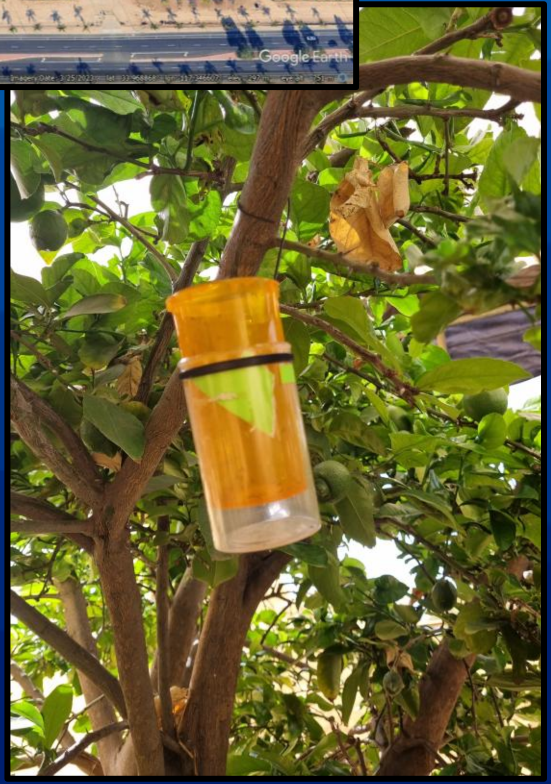
- 2-3 day-old virgin adult ACP are aspirated into secure “pharmaceutical” vials – 800 per vial.
- Vials placed inside a cooler @ 6^{o}C and driven to USDA-APHIS, Salinas overnight.
- Irradiated @ 240 Gy or 320 Gy.
- …returned to cooler and driven back to UCR.
- All movement in accordance with CA State Plant Pest Permit # 4033 and ACP remain sealed inside the secure vials throughout!



Sterile releases against "surrogate" wild populations?

- All releases in accordance with CA State Plant Pest Permit # 4033, and vials remain sealed until ready for release inside the cages!
- Field-cages sited at Agricultural Operations, UCR (10'x10'x10' or 12'x12'x12').
- Each cage housed a mature lemon tree [~7 ft high x 7 ft canopy diam.]
- Each cage seeded with 150 wild (non-irradiated) ACP.
- Vials of sterile ACP hung in the canopy and ACP allowed to naturally disperse.



Year 1 – impact of a single sterile release

- Populations initiated by releasing wild and irradiated ACP together
- Experiments conducted in Spring and Summer 2024

Treatment	Wild ACP	Irradiated ACP	No. of cages
240 Gy, 10:1	150	~1,500	4
240 Gy, 15:1	150	~2,250	4
320 Gy, 10:1	150	~1,500	4
320 Gy, 15:1	150	~2,250	4
Control	150	-	4
Total			20

Population monitoring (Yr 1):

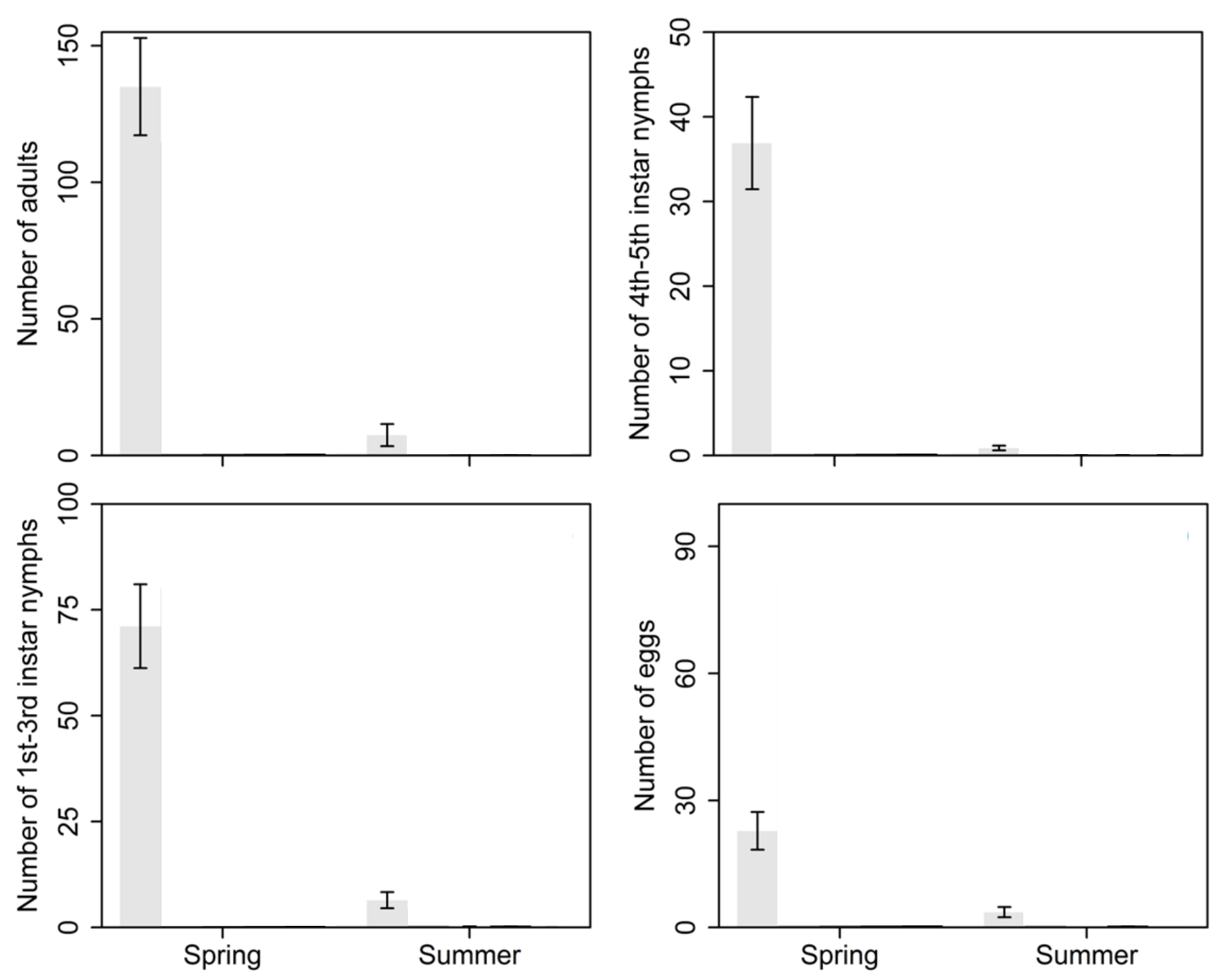
- Degree-day model for ACP (Liu & Tsai 2000) used to predict optimal sampling times for estimating the size of the F1 generation
  - Incorporating temperature data from (CIMIS #44, U.C. Riverside)

- Immature stages (eggs and nymphs) were recorded by destructive sampling of branch tips.
  - Five tips collected from each side of the tree (N, S, E, & W) – 20 total.
  - Flush stage of tip rated from 1-5 (youngest to oldest).
  - Eggs and nymphs counted under a stereo microscope.
  - Nymphal counts split into early- (1^{st}-3^{rd}) and late (4^{th}-5^{th}) instar.

- Adults sampled using yellow panel traps.

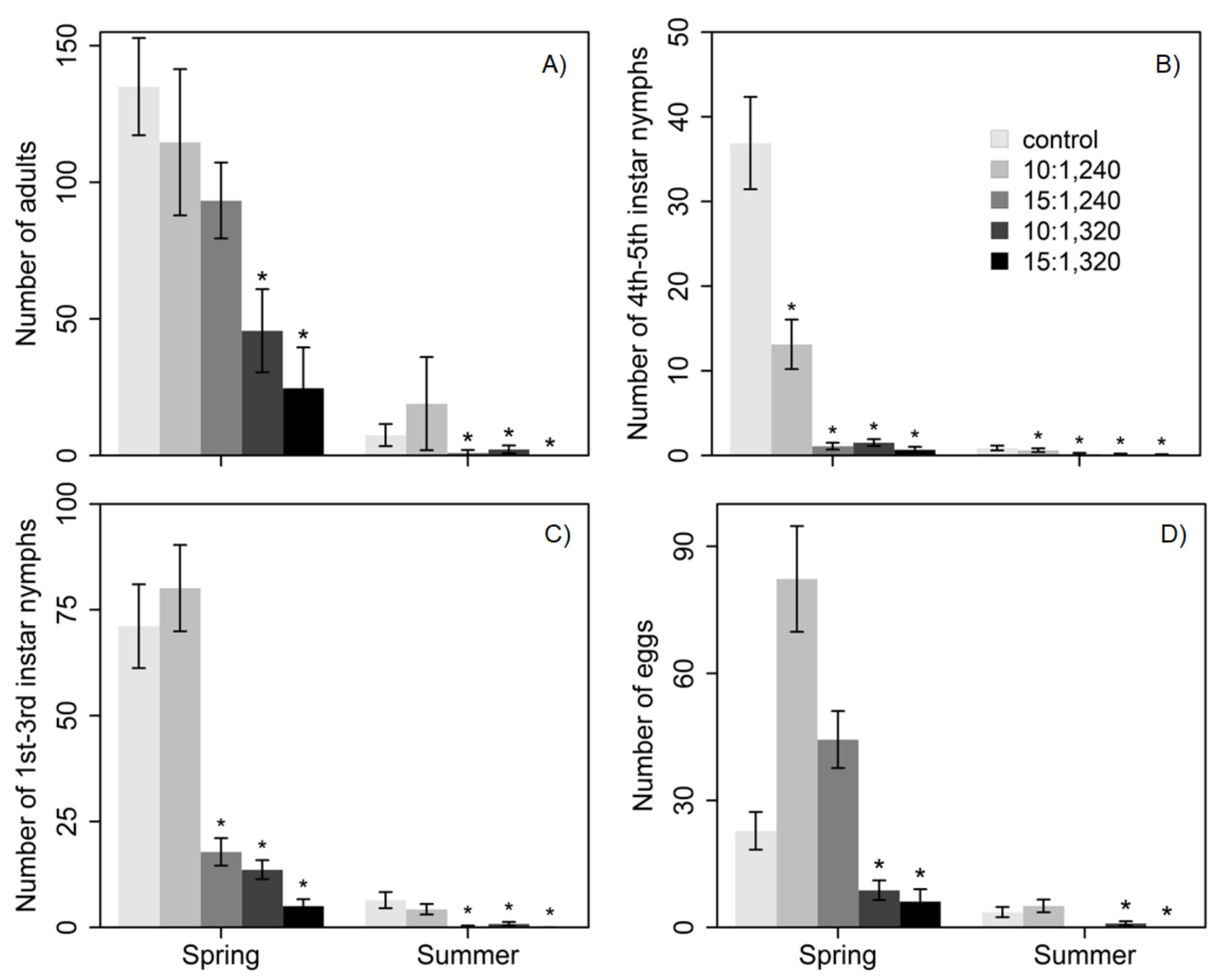
Year 1 – impact of a single sterile release

- But first - growth of the wild ACP without a sterile release?



Year 1 – impact of a single sterile release

- ACP populations lower in the summer, but impact consistent across seasons.
- In general, increasing the radiation dose (from 240 Gy to 320 Gy) showed stronger suppression than increasing the OFR (from 10:1 to 15:1).
- Combination of 320 Gy and 15:1 OFR reduced ACP counts the most compared to the control:
  - A. >80% for adults
  - B. ~98% reduction in late-instar nymphs
  - C. >90% reduction in early-instar nymphs
  - D. ~80% reduction in eggs,



	Eggs			1st-3rd instars			4th-5th instars			adults		
Source	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P
Treatment	38.864	4	<0.0001	60.161	4	<0.0001	57.547	4	<0.0001	31.889	4	<0.0001
Season	201.54	1	<0.0001	301.04	1	<0.0001	137.40	1	<0.0001	78.829	1	<0.0001
Flush	45.812	1	<0.0001	43.105	1	<0.0001	6.662	1	0.0098	--[1]	--	--
Trt*Seasn	--[2]	--	--	--	--	--	--	--	--	11.654	4	0.0201

Year 2 – impact of multiple sterile releases

- ACP prepared and irradiated as Yr 1 – 320 Gy only
- Populations initiated by releasing wild and irradiated ACP
- Two OFRs [10:1 & 15:1]
- Half the cages only a single sterile release (same as Yr 1)
- Other half, two further sterile releases after 7 & 14 days
- Experiments conducted in Spring and Summer 2025

Treatment	Wild ACP – Wk1	Irradiated ACP – Wk1	Wk2	Wk3	No. of cages
10:1, single	150	~1,500	-	-	4
15:1, single	150	~2,250	-	-	4
10:1, multiple	150	~1,500	~1,500	~1,500	4
15:1, multiple	150	~2,250	~2,250	~2,250	4
Control	150	-	-	-	4
Total					20

Population monitoring (Yr 2):

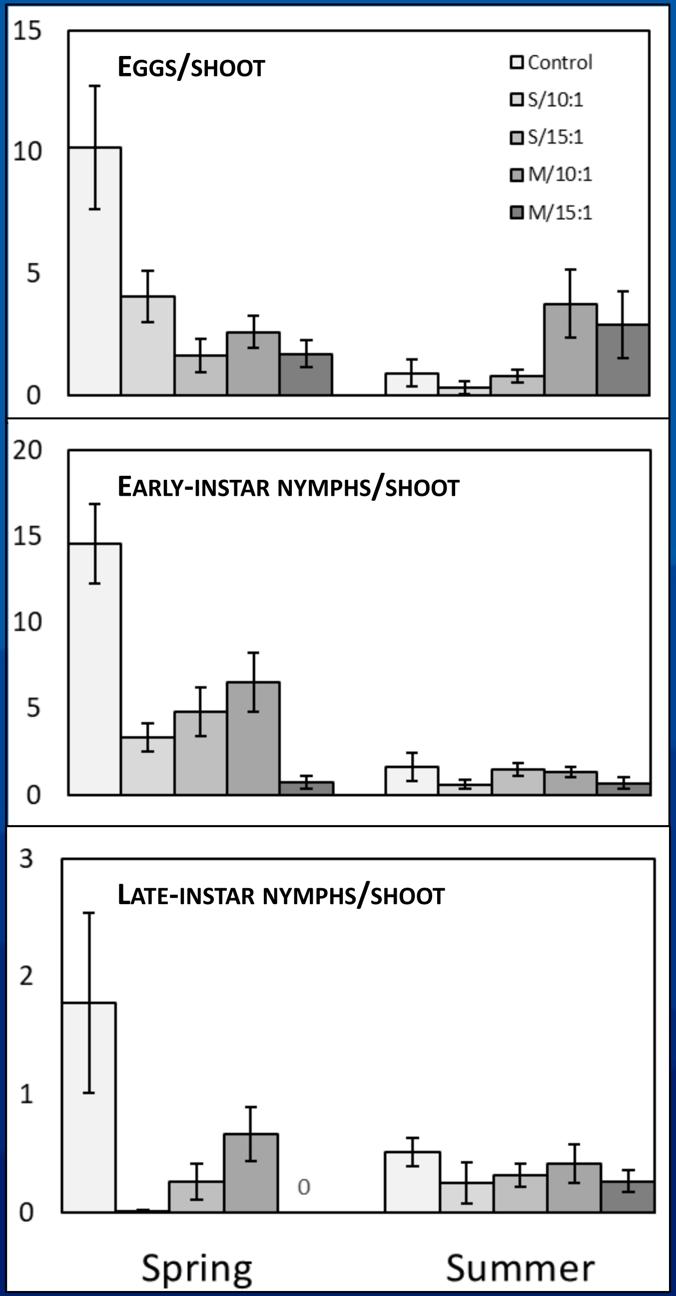
- Degree-day model for ACP (Liu & Tsai 2000) used to predict optimal sampling times for estimating the size of the F1 generation
  - Incorporating temperature data from (CIMIS #44, U.C. Riverside)

- Immature stages (eggs and nymphs) were recorded by destructive sampling of branch tips.
  - Five tips collected from each side of the tree (N, S, E, & W) – 20 total.
  - Flush stage of tip rated from 1-5 (youngest to oldest).
  - Eggs and nymphs counted under a stereo microscope.
  - Nymphal counts split into early- (1^{st}-3^{rd}) and late (4^{th}-5^{th}) instar.

- Adults not sampled at the F1 generation.

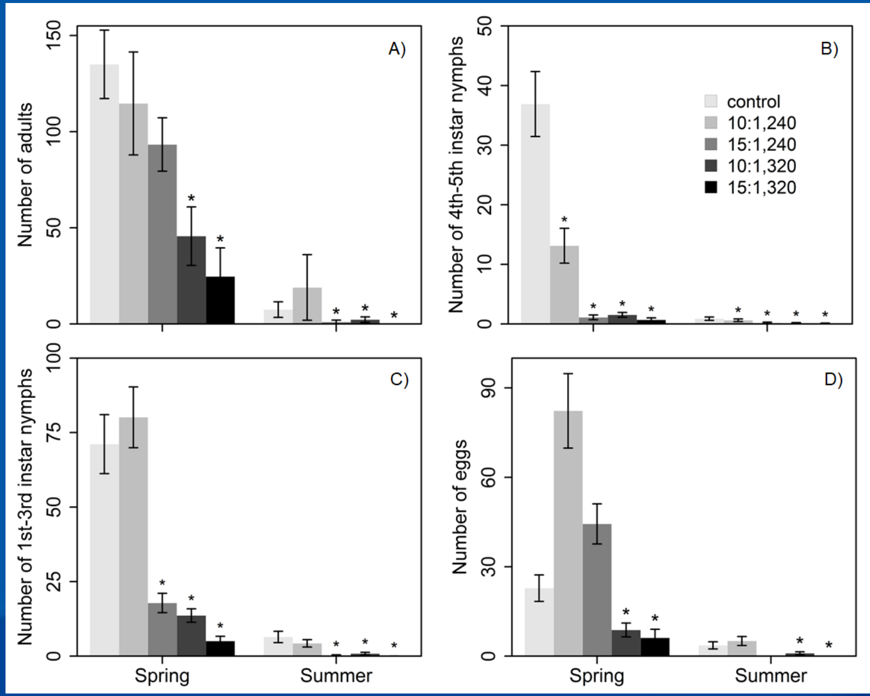
Year 2 – impact of multiple sterile releases

- ACP populations again lower in the summer
- But control cages also >5-fold lower population counts than in Year 1.
- Summer counts particularly low!
- Though overall not statistically significant, sterile releases had the expected impact on the caged populations in the spring.
- Largest suppressive effect brought about via multiple spring releases of sterile ACP at 15:1 OFR.



Summary & future directions

- ACP can be mass-reared and sterilized
- With the right combination of dose (320 Gy) and OFR (15:1), the growth of ACP populations on mature citrus trees held within field-cages can be significantly suppressed with the release of sterile psyllids.
- Caged experiments are closed-trials – preventing continued recruitment of wild ACP, and emigration of both sterile and wild ACP from the population.
- We also took steps to exclude natural enemies.
- How will sterile ACP perform in an open environment?

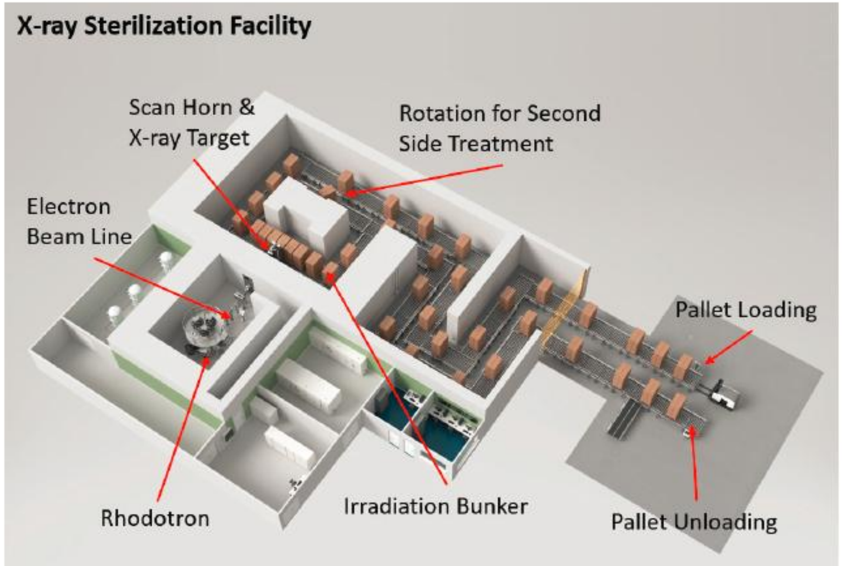


Challenges - infrastructure

- Mass-rearing
  - Investment in rearing facilities/ greenhouse?
  - Can we develop an artificial diet for rearing?

Photo: Farm Progress

- Sterilization
  - Capacity?



Rhodotron® TT1000 x-ray facility. SOURCE: IBA Industrial

Photo: Cal Poly Pomona

Thank you!