Addressing whether there is a need for continued vector management under HLB stress

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Do insecticides sprays affect yield in trees that are already infected with HLB?

Objective: Evaluate effect of insecticides and foliar nutrition on ACP, HLB incidence, and yield

- 13 acres 'Valencia' on 'Swingle'
- Planted June 2001
- Defoliated 2005 and 2006 for canker control
- HLB detected spring 2006
- 2 x 2 factorial (RCBD 4 reps)
  - 16 plots
  - Average 108 trees per plot
  - Treatments began Feb 2008

	No- Insecticide	Insecticide
No-Nutritional	Control	Insecticide
Nutritional	Nutritional	Nutritional + Insecticide

Stansly, P.A., Arevalo, H.A., Qureshi, J.A., Jones, M.M., Hendricks, K., Roberts, P.D. and Roka, F.M., 2014. Vector control and foliar nutrition to maintain economic sustainability of bearing citrus in Florida groves affected by huanglongbing. Pest management science, 70(3), pp.415-426.

Tansey, J.A., Vanaclocha, P., Monzo, C., Jones, M. and Stansly, P.A., 2017. Costs and benefits of insecticide and foliar nutrient applications to huanglongbing-infected citrus trees. Pest management science, 73(5), pp.904-916.

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Cumulative of ACP adults per tap

- **Two taps/tree**
- **2008-2010:** 10 randomly selected trees in the middle bed of each plot
- **2010-2014:** 6 randomly selected trees per two randomly selected stops per plot

Sprays (Avg 5/year) dormant + threshold based on 0.5,0.2 and finally 0.1 ACP/TAP

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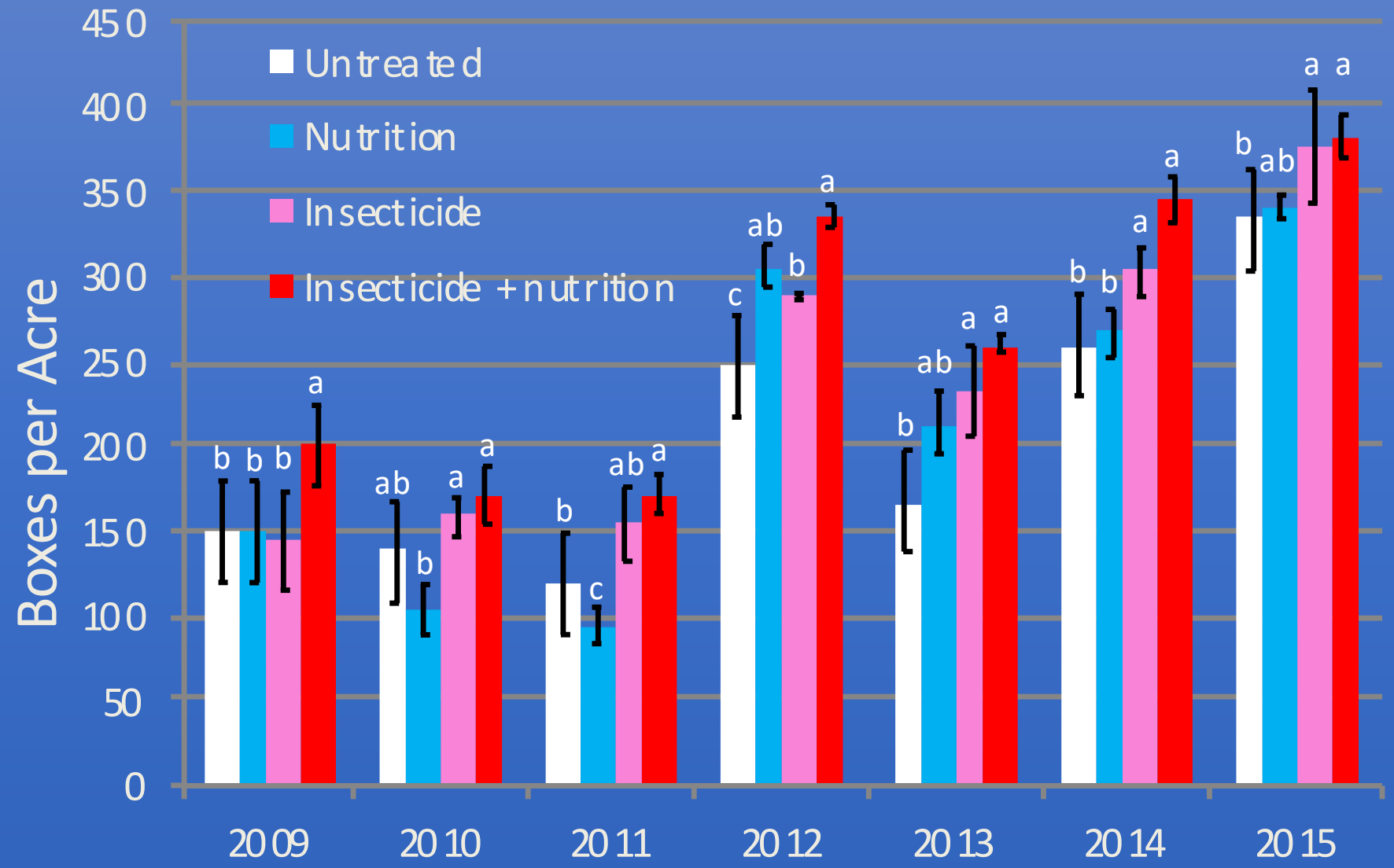

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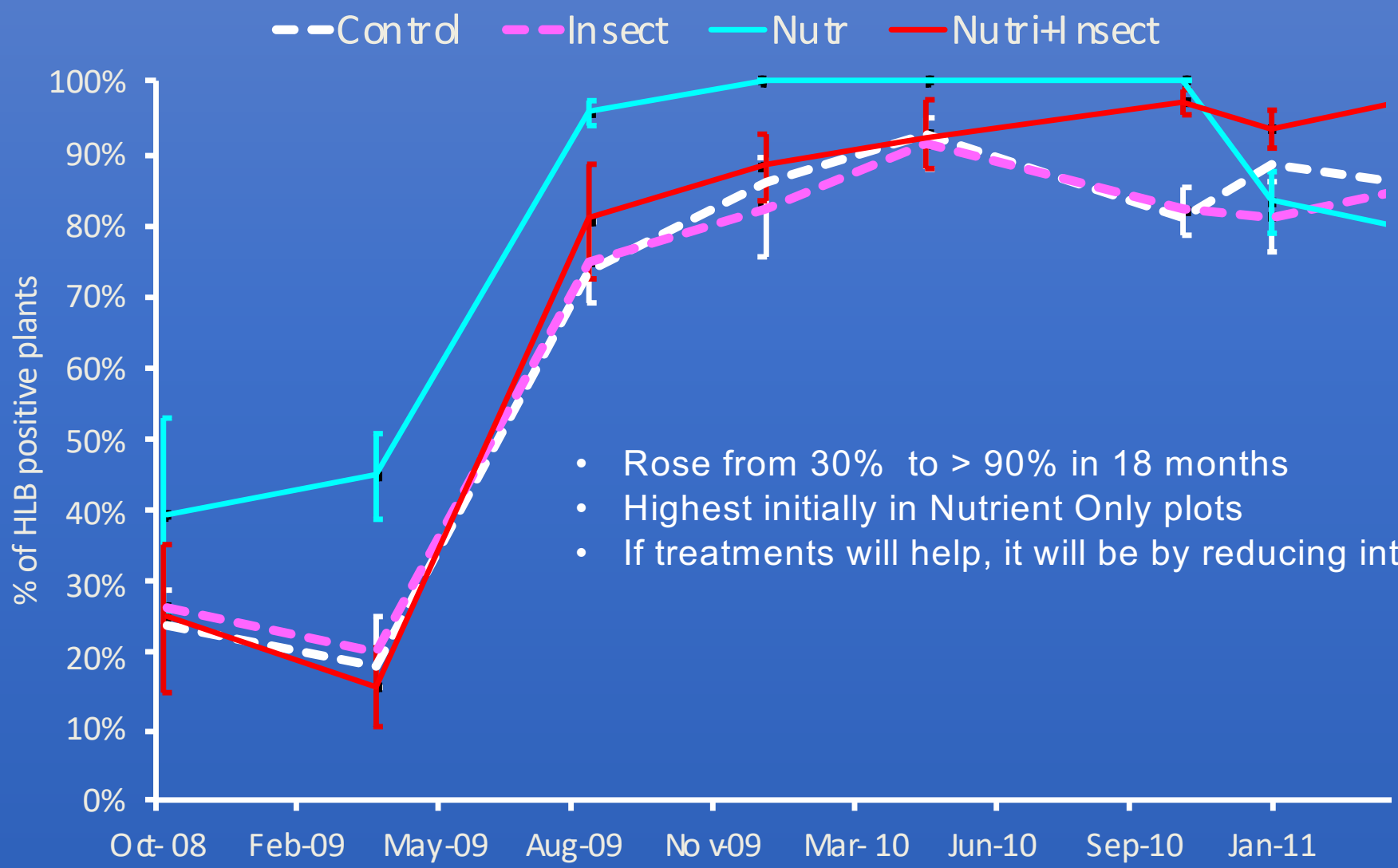
Incidence of HLB: Assessed by PCR on 20% of trees per treatment



- Rose from 30% to > 90% in 18 months
- Highest initially in Nutrient Only plots
- If treatments will help, it will be by reducing intensity of HLB

Phil Stansly

Project Title: Addressing whether there is a need for continued vector management under HLB stress

- USDA, APHIS Multi-Agency Coordinating Group (MAC)
- PIs: L. Stelinski (UF) and M. Setamou (Texas A&M)
- Project initiated in January 2018; Initial funding duration 2 yrs.

Laboratory experiments

Evaluate the effect of varying *C*Las inoculation frequencies on HLB progression and plant defense response

Specific measurements

1. *C*Las titer within various plant tissues (mature leaves, flush feathers and roots)

1. Salicylic acid-induced plant defense response

2. Disease progression

Hypotheses

H_1:'**Suppression** of ACP populations in citrus groves with **improved** vector management will reduce CLas titer in citrus trees via decreased **super-infection**'

H_2: Decreasing super-infection will decelerate decline

CLas-Infected

ACP populations

Improved vector suppression

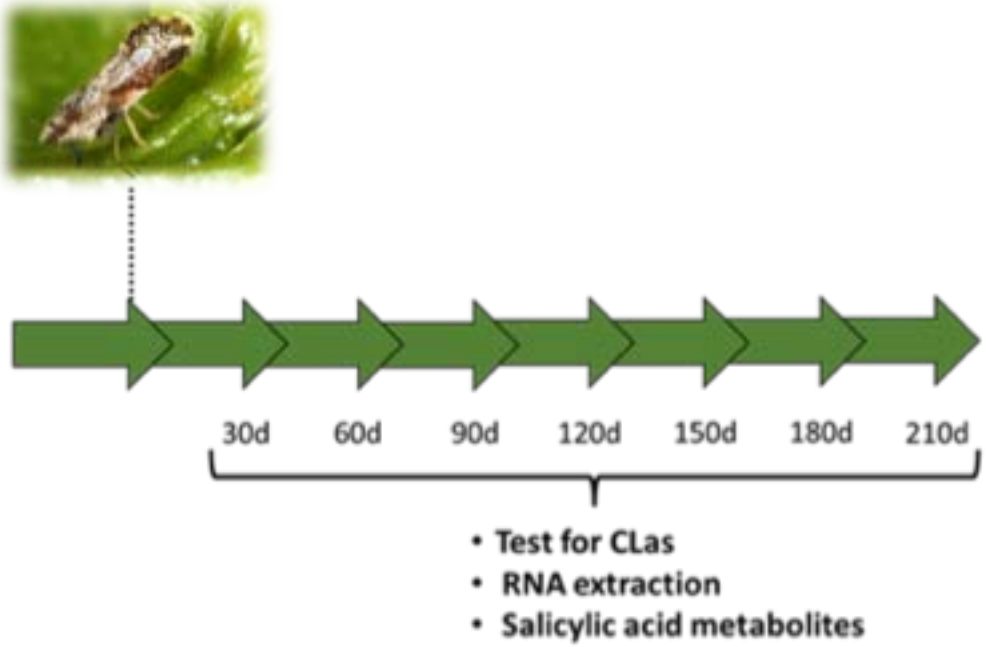
CLas-super-infected
Accelerated decline

Experimental design (laboratory microcosm)

- One-time inoculation: Testing the ‘all it takes is a single infection’ hypothesis. Do we need zero-tolerance?

*C*Las-infected ACP

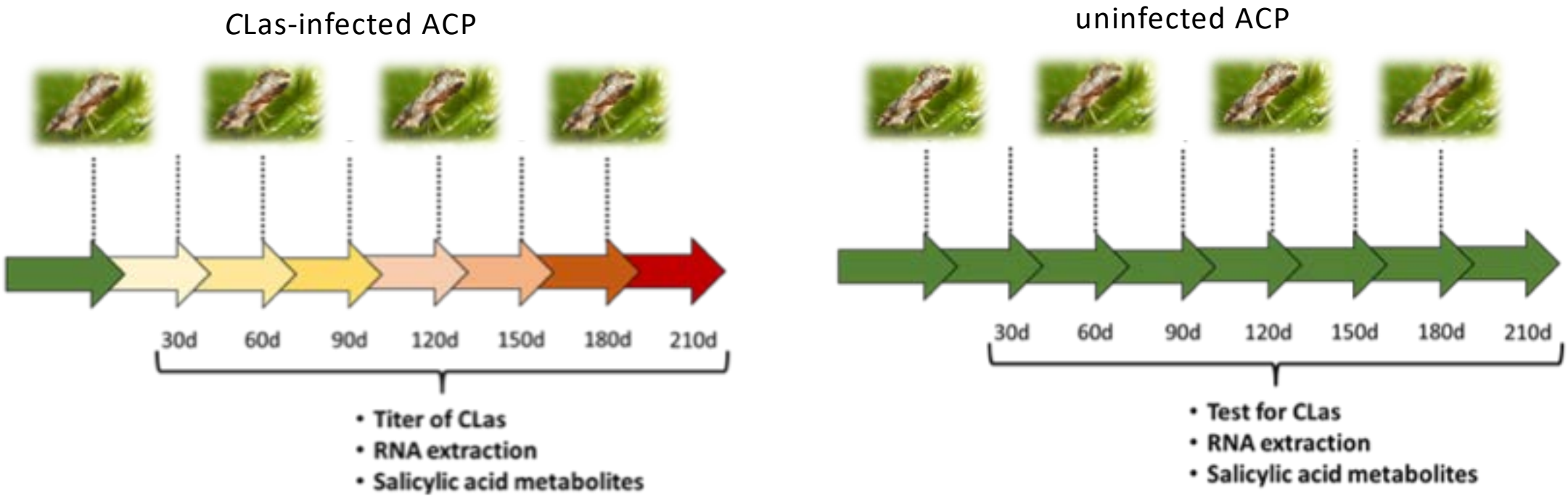
Uninfected ACP (Negative control)



Plants are exposed to adult ACP (*C*Las infected vs. uninfected) allowing 7 d inoculation period.

Experimental design (laboratory microcosm)

- Regularly pulsed inoculations (Simulating ACP control followed by invading immigrants)

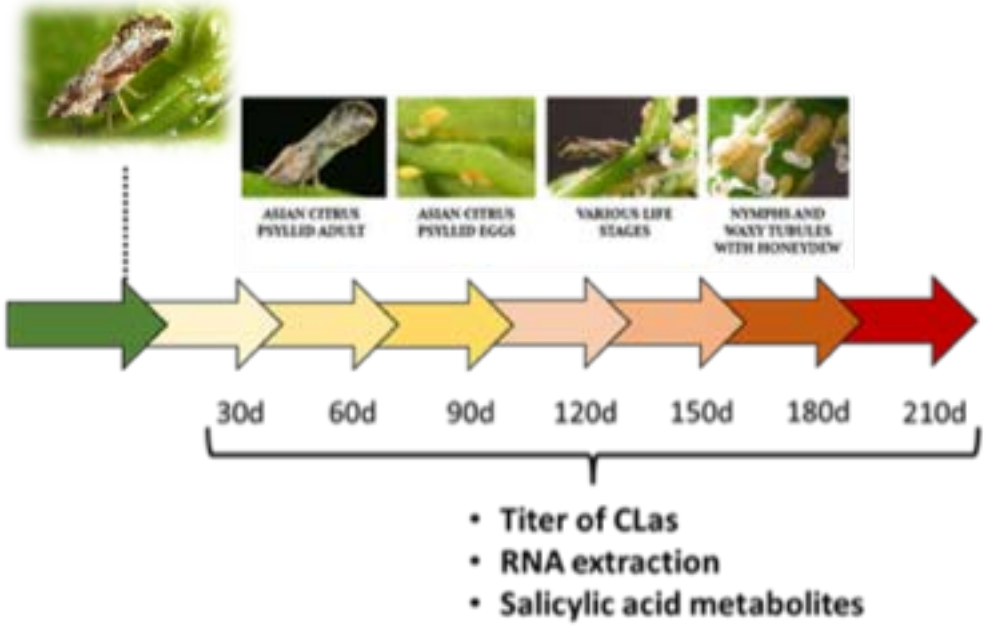


Plants are exposed to adult ACP (*C*las-infected vs. uninfected) every 30 days (monthly) for an inoculation period of 7 days. This process is continuously repeated.

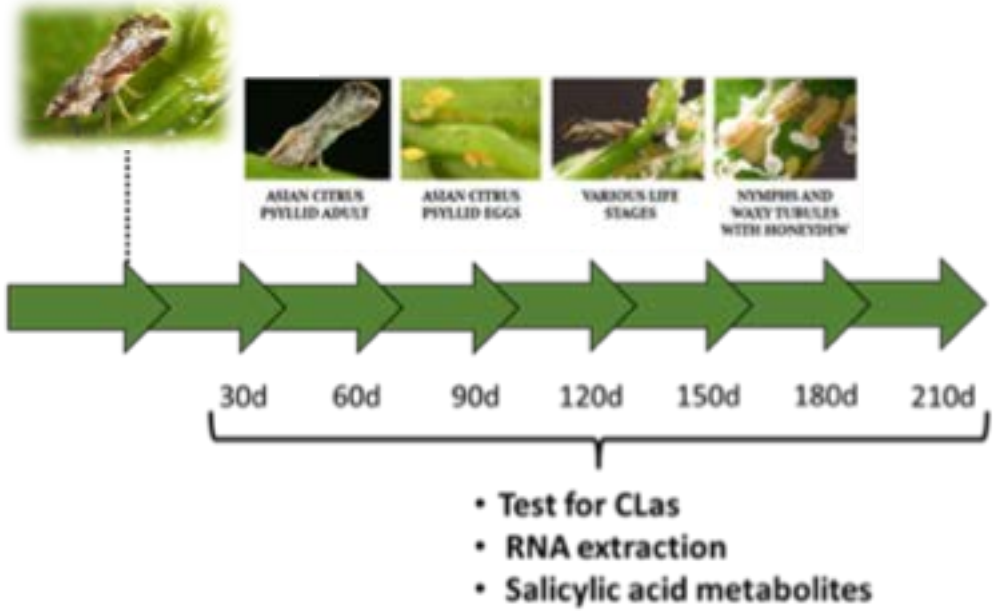
Experimental design (laboratory microcosm)

- Continuous inoculation (Simulating constantly reproducing resident population of ACP)

CLas-infected ACP



uninfected ACP



Plants are exposed to all life stages of ACP (*C*Las infected vs uninfected infected) continuously during the entire experiment.

One-time inoculation:

Pulsed inoculation:

Continuous inoculation:

‘Hot’ ACP

Uninfected (control) ACP

Preliminary results from laboratory microcosms

Summary of CLas titer, plant phenotype and gene expression results:

-No symptoms or *C*Las infection among negative control plants exposed to uninfected ACP or not exposed to ACP at all.

Treatments	Percentage of CLas-infected plants (3 months after inoculation)	HLB symptoms	Genes involved in SA-pathogen response and metabolism
One-time inoculation	33%	**+**	up-regulated
Multiple inoculations	0%	**++**	up-regulated
Extended inoculations	0%	**+++**	up-regulated

Abbreviations: (+) normal leaves, (++) mature leaves with yellowing, (+++) mature and young leaves are deformed, stunted in growth with yellowing.

-SA response upregulated by ACP feeding irrespective of whether they were carriers of *C*Las.

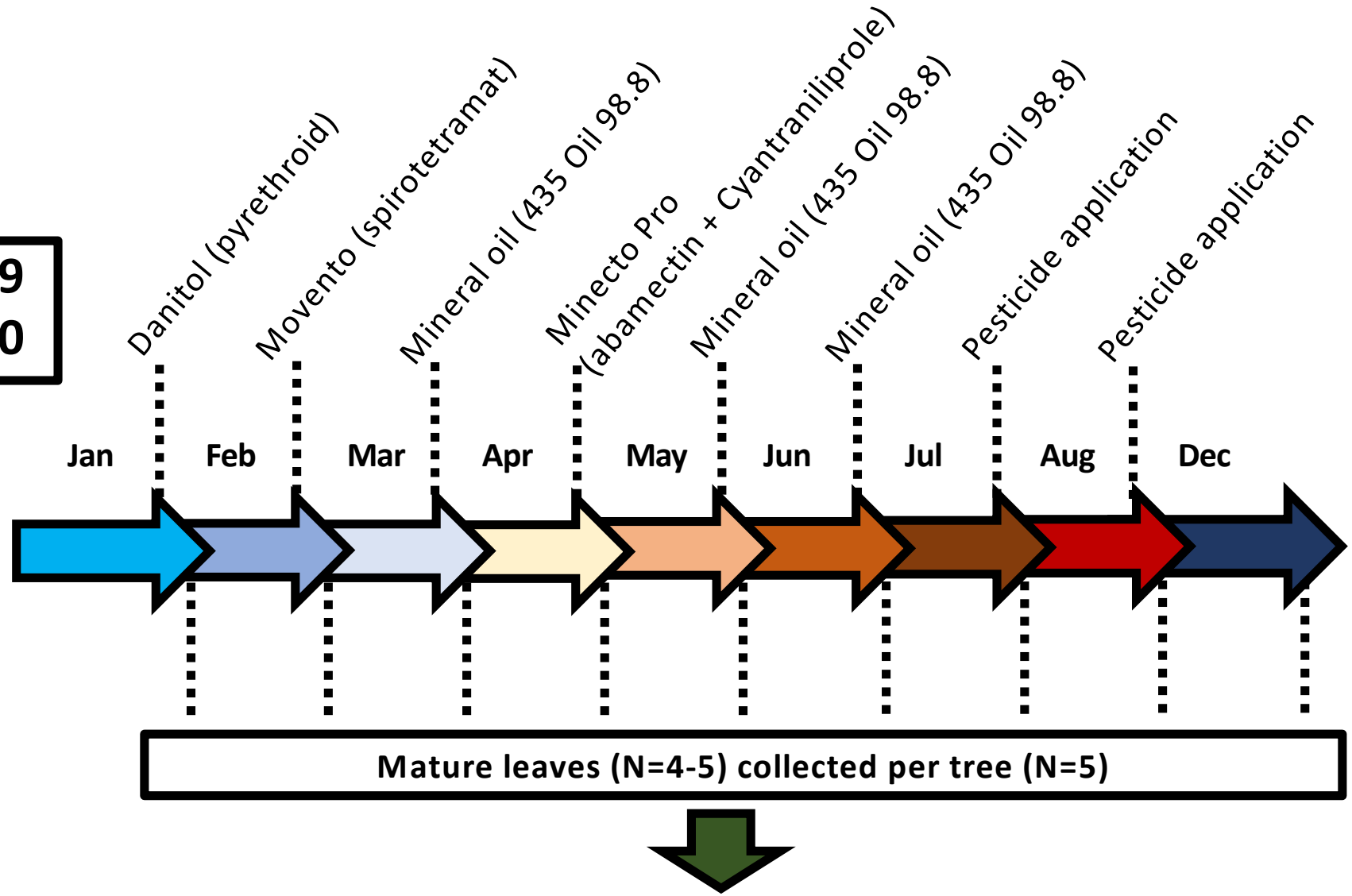
Field experiment: Regular interval sprays versus sprays timed to tree (ACP lifecycle) phenology

- ‘Control’ consists of calendar sprays: Intervals between sprays are determined by length of treatment efficacy and dormant sprays and the first spray thereafter often timed to first flush.

- ‘Treatment’ is the Dr. Gene Albrigo method: Spray for adults at bud break at the beginning of each new flush before there is feather flush on which the adults can lay eggs. With a second spray as ACP begins to reappear—> 60 days of low ACP populations seem possible.

Experimental design for Field expeirment

2018-2019
2019-2020

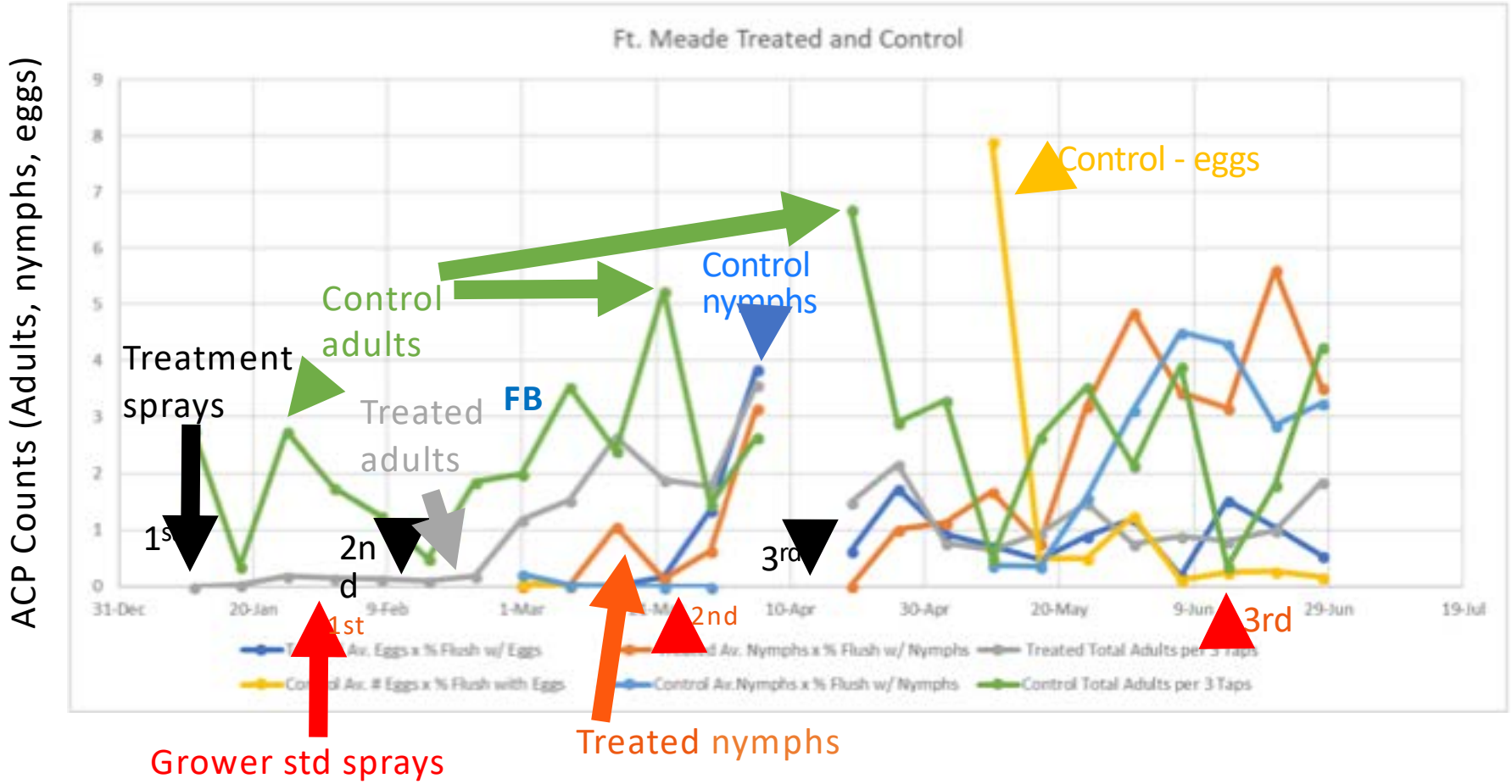


Locations

1. Fort Meade L1
2. Fort Meade L2
3. Fort Meade L3
4. Frostproof L4
5. Frostproof L5
6. Frostproof L6

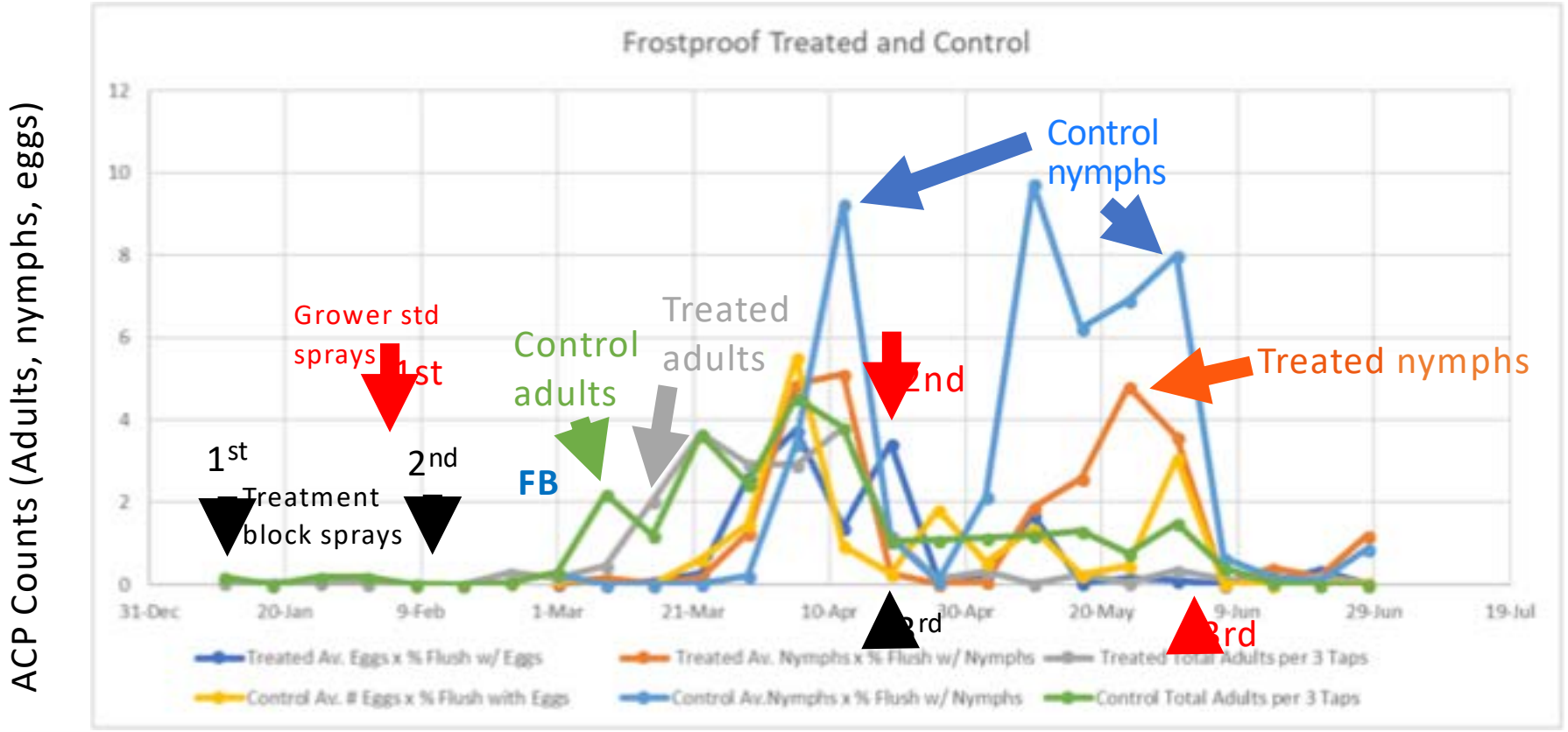
- **Total number of ACP (taps)**
- **Titer of CLas (DNA extractions)**

Initial results-ACP counts



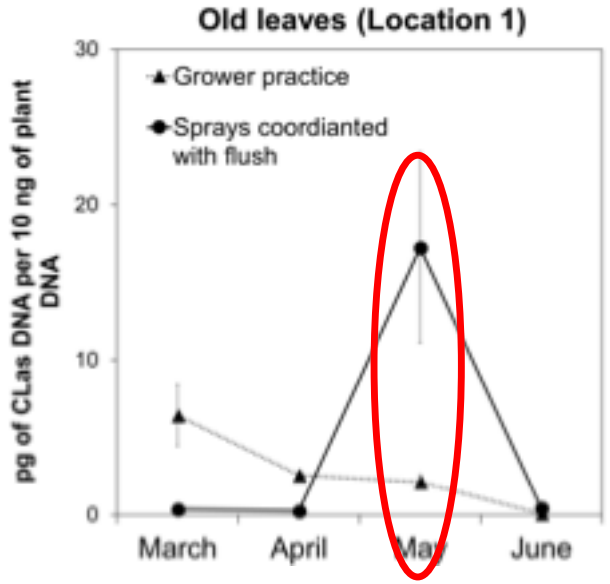
Treatment: 1/16 Danitol, 2/12 Movento + oil, 4/12 Movento + Malathion

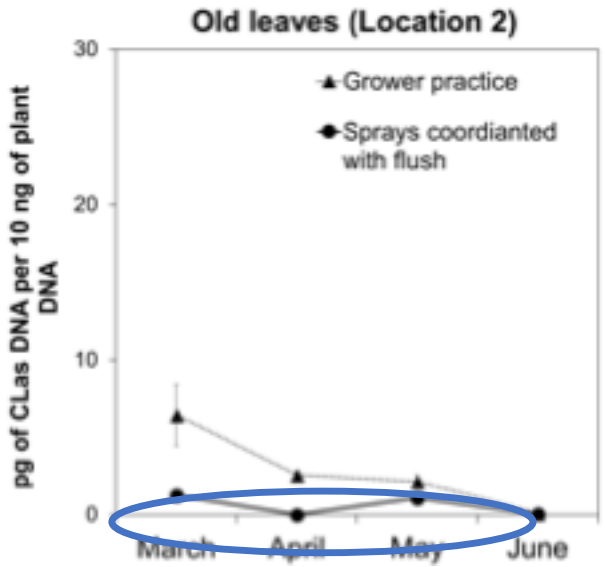
Initial results-ACP counts

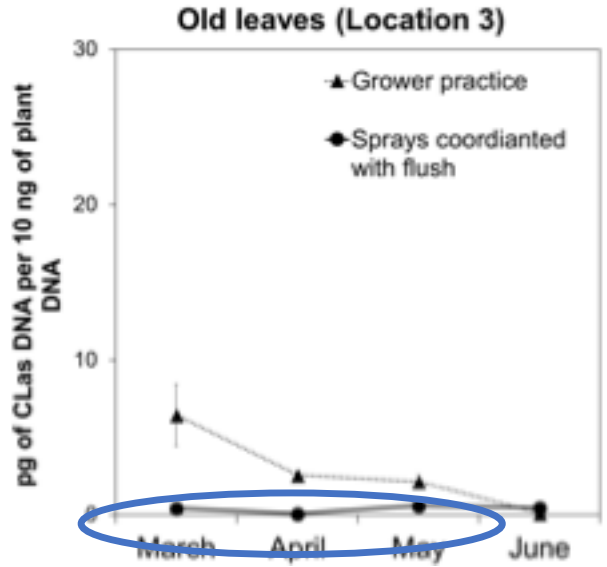


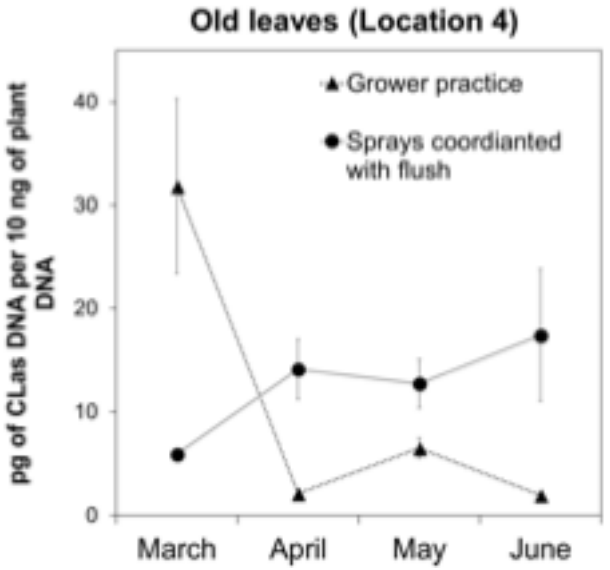
1/16 Danitol, 2/12 Movento + oil, 4/12 Movento + Malathion

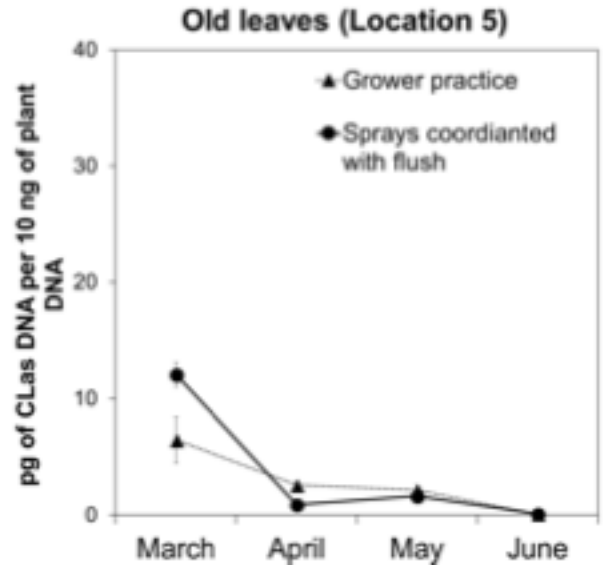
Initial CLas titers measured to date

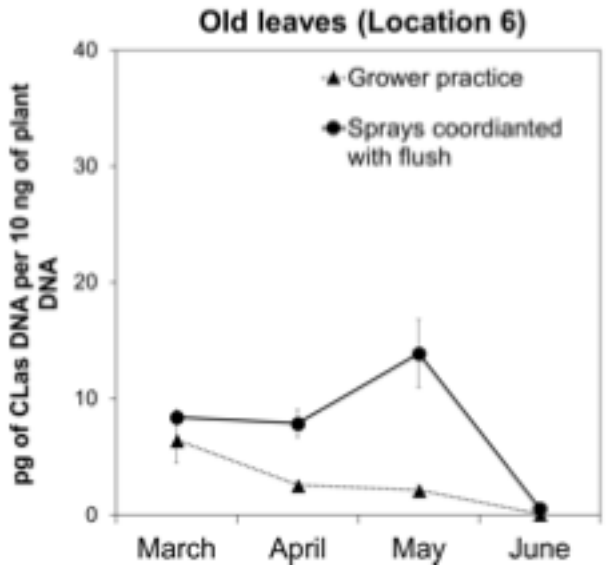












Conclusions

- Vector management with Gene Albrigo method of timing sprays with bud break reduced ACP more effectively than non-targeted calendar sprays.

- We **expect** to observe a reduction in CLas titer associated with a suppression of ACP populations during the previous season's feather flush. Whether reducing super-infection will cause decreased CLas titer associated with improved health is to be determined.

- Are insecticides worth it?
  - **The preponderance of available data supports this hypothesis**
  - Has this new MAC-funded investigation produced unequivocal supporting data? Not yet; work is in progress

Acknowledgments

CRDF funded previous work – P. Stansly

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