Agenor Mafra-Neto (ISCA Technologies Riverside); **David Hall** (ARS Ft. Pierce); **Stephen Lapointe** (ARS Ft. Pierce); **Joseph Patt** (ARS Ft. Pierce); **Paul Robbins** (ARS Ft. Pierce); **Mamoudou Sétamou** (TAMU, Weslaco); **Lukasz Stelinski** (UF Lake Alfred)

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Pest Management Tools & Solutions

Outcome Group 2, Topic 3:

Chemical Ecology of The Asian Citrus Psyllid

ISCA Technologies, Inc.
1230 Spring Street
Riverside CA 92507 USA
www.iscatech.com

ISCA Technologies, Inc.
3rd Annual Citrus Health Research Forum, Ft. Collins, CO
Outcome Group 2; Topic 3
Date: 08/28/2012

Applied Chemical Ecology of ACP

- Better Understand the Ecology and Chemical Ecology of the interactions between **Citrus/ACP/Beneficial/HLB**
  - Semiochemicals to manipulate the behavior of
    - ACP vector
    - Beneficials / Natural Enemies
    - Citrus host plants
  - Integrate Novel Techniques Into the Pest Management Protocols

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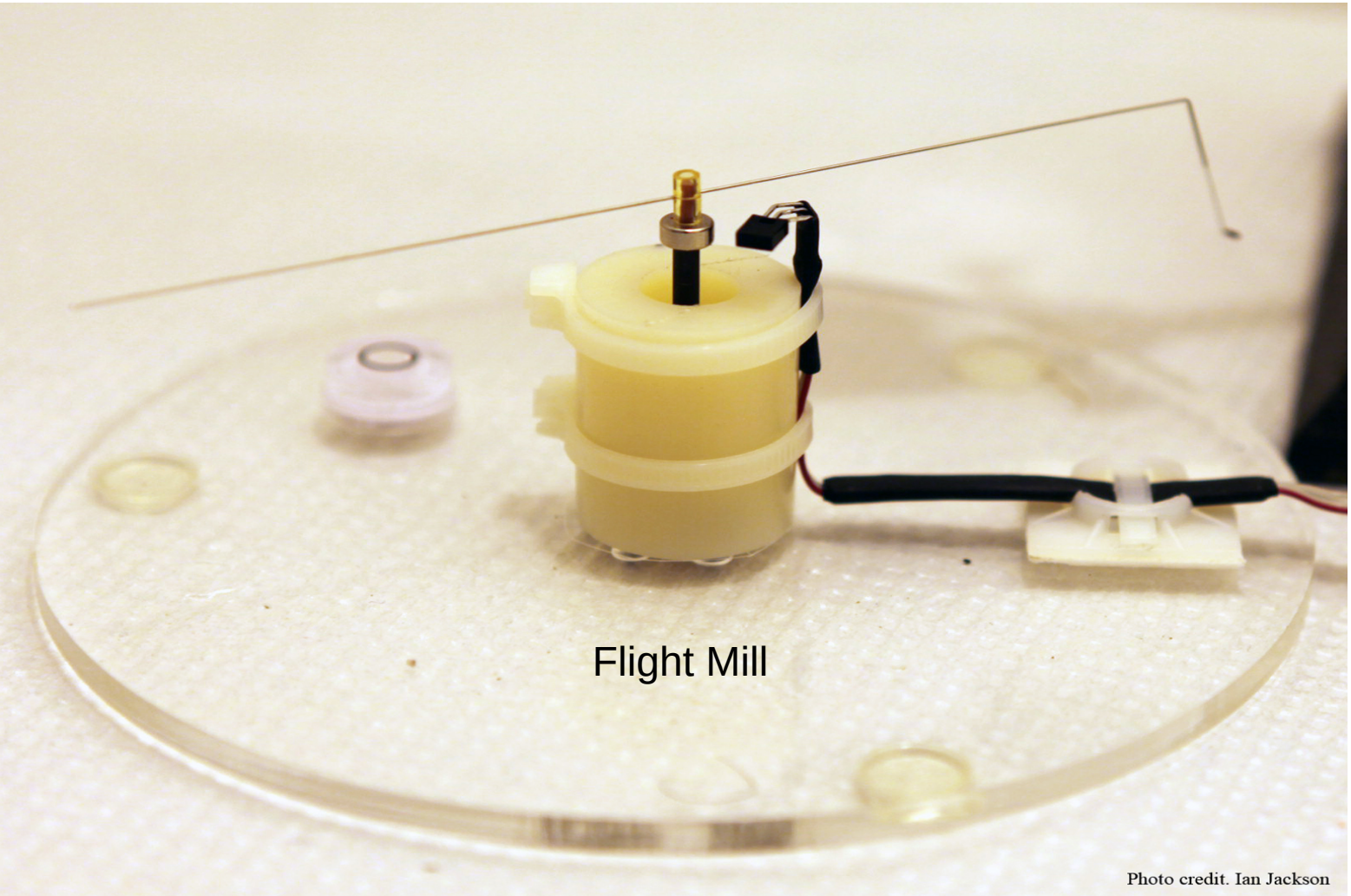
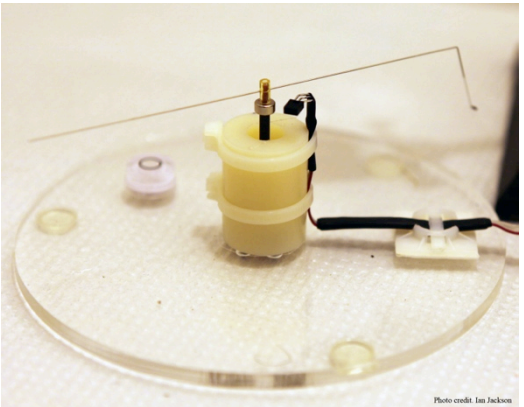
How Far Can Psyllids Move on Their Own?

Stelinski



How Far Can Psyllids Move on Their Own?

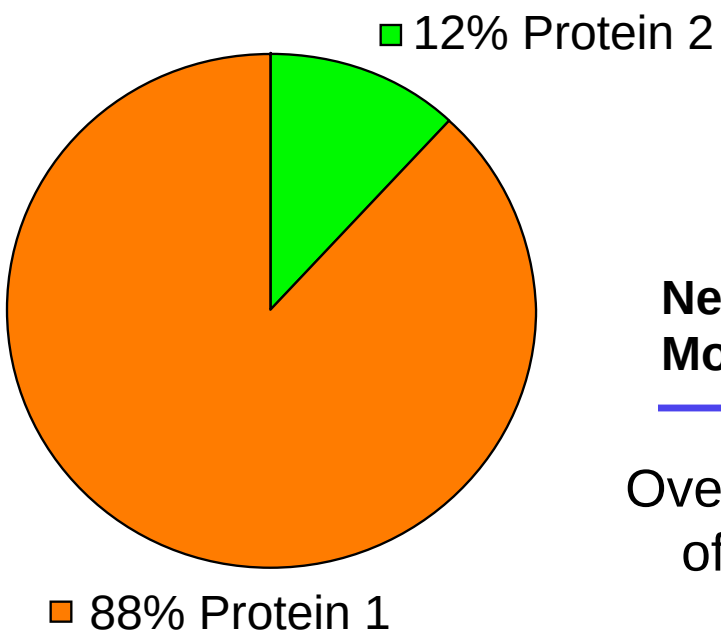


- Capable of 50 minutes of continuous flight
- Capable of nearly one mile of continuous flight

ACP Movement is Biased Toward Managed Groves When Unmanaged and Managed Groves are Adjacent

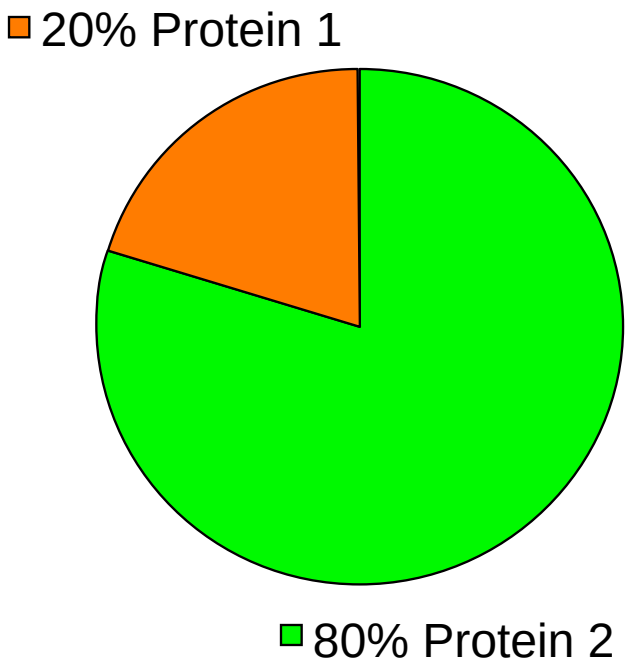
Stelinski

Percentage of captured ACP marked with protein markers



Net ACP Movement

Over the course of two days



Unmanaged Grove marked with Protein 1

Managed Grove marked with Protein 2

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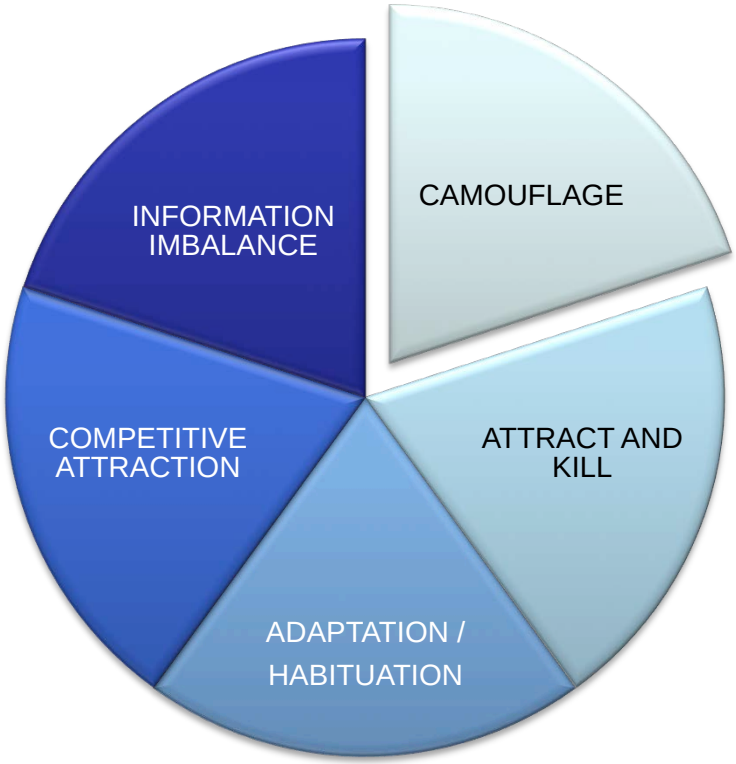
ACP Aggregates on the Borders of the Field

Can We Disrupt the Behavior of ACP?

MATING DISRUPTION
We have experience with Lepidoptera and Coleoptera

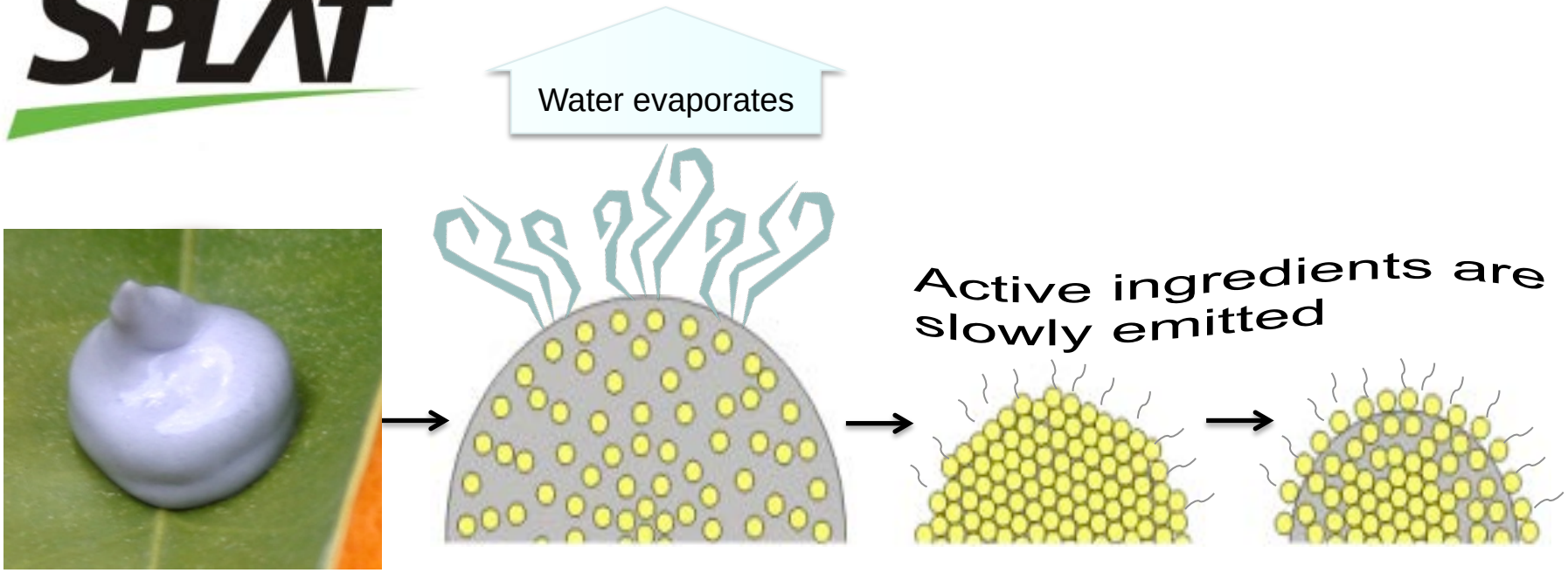


These mechanisms can act individually or together, separately or sequentially, promoting a stronger effect in some situations or a weaker in others.

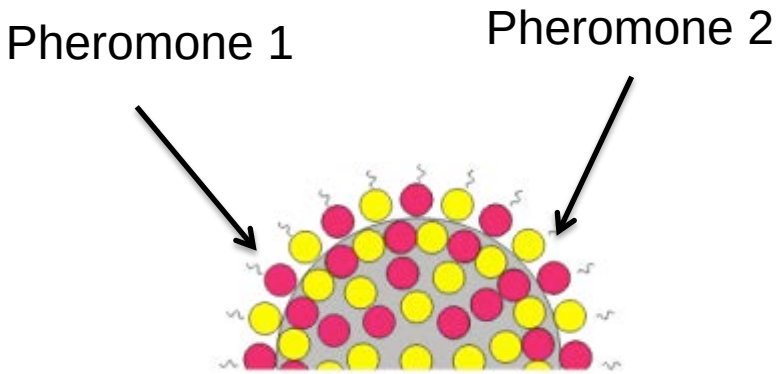
Specialized Pheromone & Lure Application Technology

SPLAT has many desirable qualities as a controlled release formulation:

- Provides rain and UV protection for pheromone and/or pesticide
- Biologically inert and bio-degradable matrix
- Possible to develop formulations to target more than one pest with formulation mix of desired pheromones and/or pesticides
- Ability to vary release rates and duration from weeks to months with one application (some formulations can achieve season long control with one application)
- Amorphous and flowable quality allows for hand application and many types of mechanical application methods: caulk gun, metered back pack sprayer, tractor, aircraft, etc.

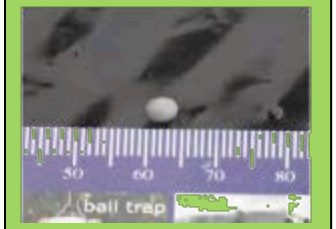


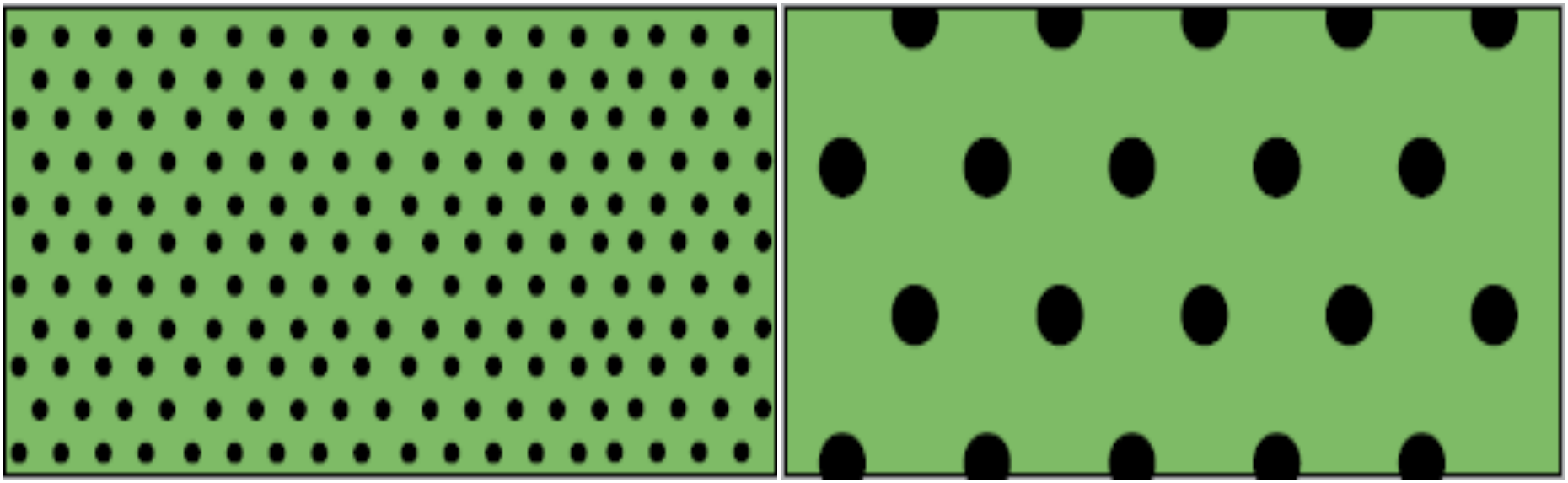
Once applied in the field, water in SPLAT begins to evaporate and the dollop shrinks, better adhering to the application surface. Once the dollop dries, molecules of active ingredients slowly migrate to the surface of the dollop, providing remarkably constant emission over long periods of time.



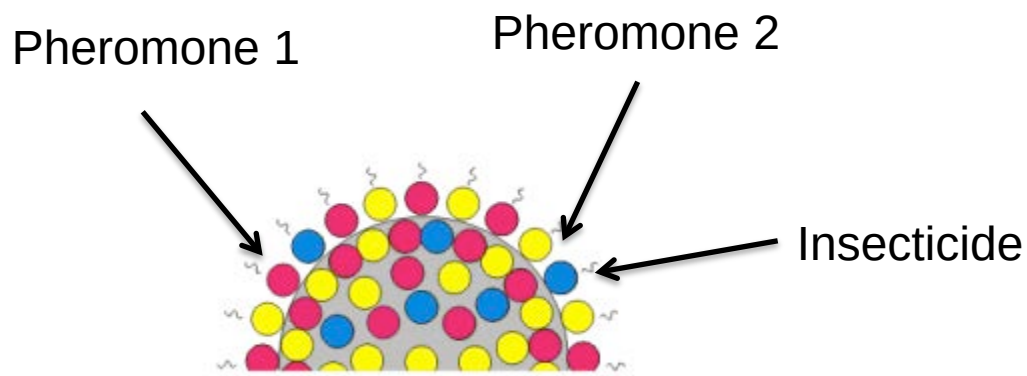
- The SPLAT matrix permits the mixture of more than one pheromone while preserving the chemical characteristics of each.

Methods of Application

Hand Apply	Airplane	Tractor	Paintball	Metered Sprayer	Caulking Gun
					



For areas with high pest pressure, the number of point sources can be increased while the size of each dollop of material is decreased (left). For lower pest densities, the number of point sources can be decreased, while the dollop size is increased (right). In both scenarios, the amount of active ingredient applied remains unchanged.



- The SPLAT matrix permits the mixture of more than one pheromone while preserving the chemical characteristics of each.
- By adding an insecticide to the SPLAT matrix, we can create a powerful, species-specific attracticide.

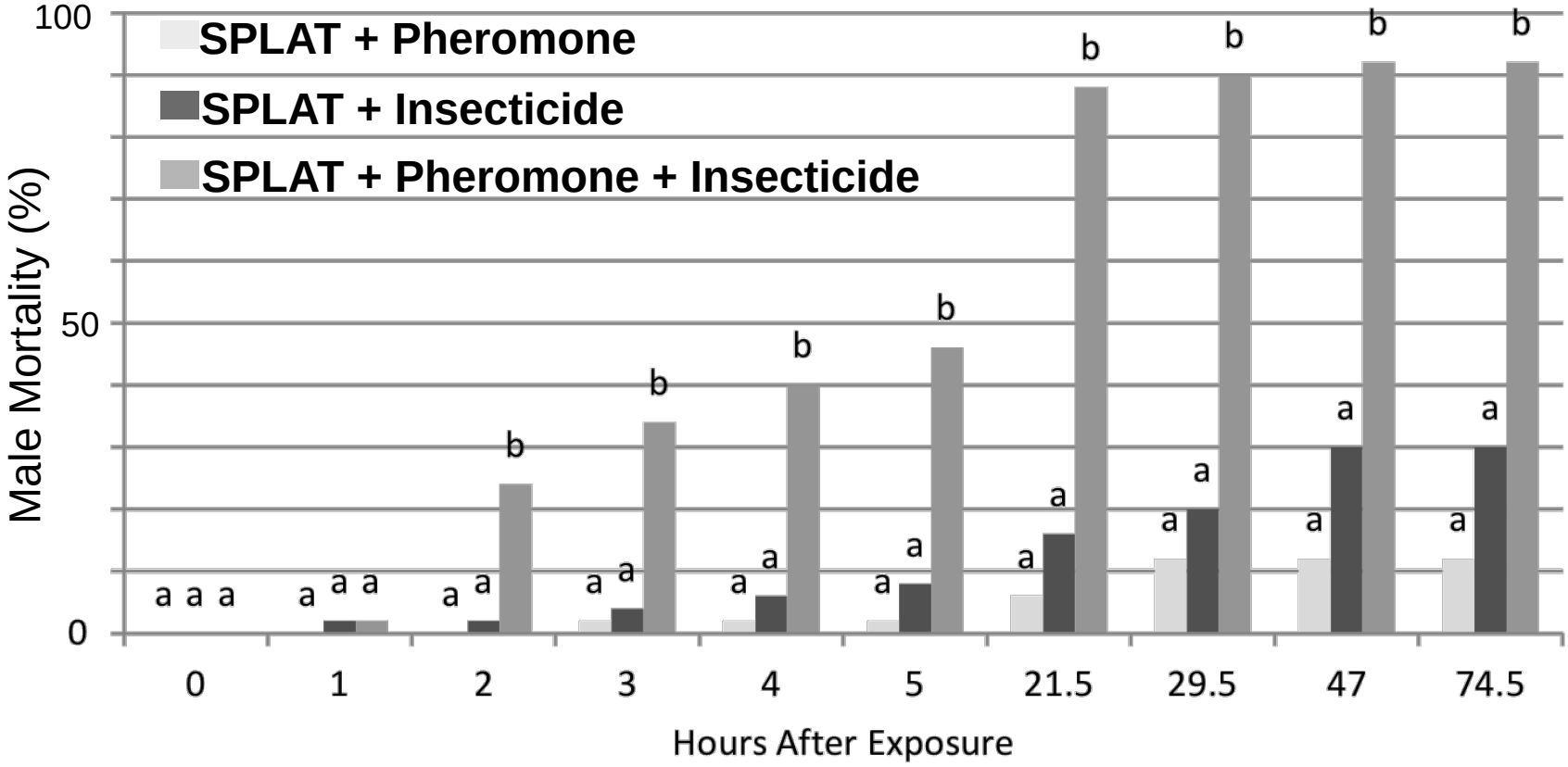
SPLAT A&K

SPLAT

- Average mortality of adult males after two minutes of exposure to a 1 ml dollop
  - SPLAT + Pheromone
  - SPLAT + Insecticide
  - SPLAT + Pheromone + Insecticide

  - Data on the mean moth mortality by treatment was analyzed separately for each sampling period.
  - Treatments within each sampling period (hours after exposure) followed by the same letter are not significantly different (ANOVA, $\alpha = 0.05$, $p < 0.0001$)

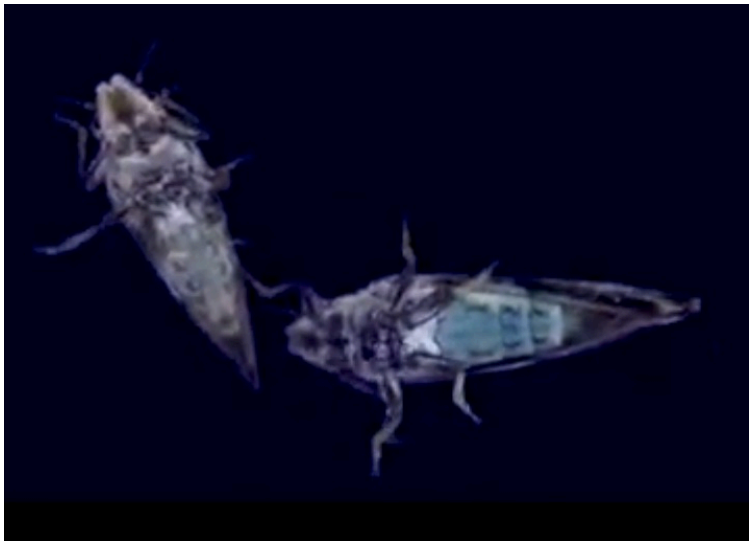
SPLAT A&K


SPLAT + Pheromone
SPLAT + Insecticide
SPLAT + Pheromone + Insecticide
Male Mortality (%)
100
50
0
0
1
2
3
4
5
21.5
29.5
47
74.5
Hours After Exposure

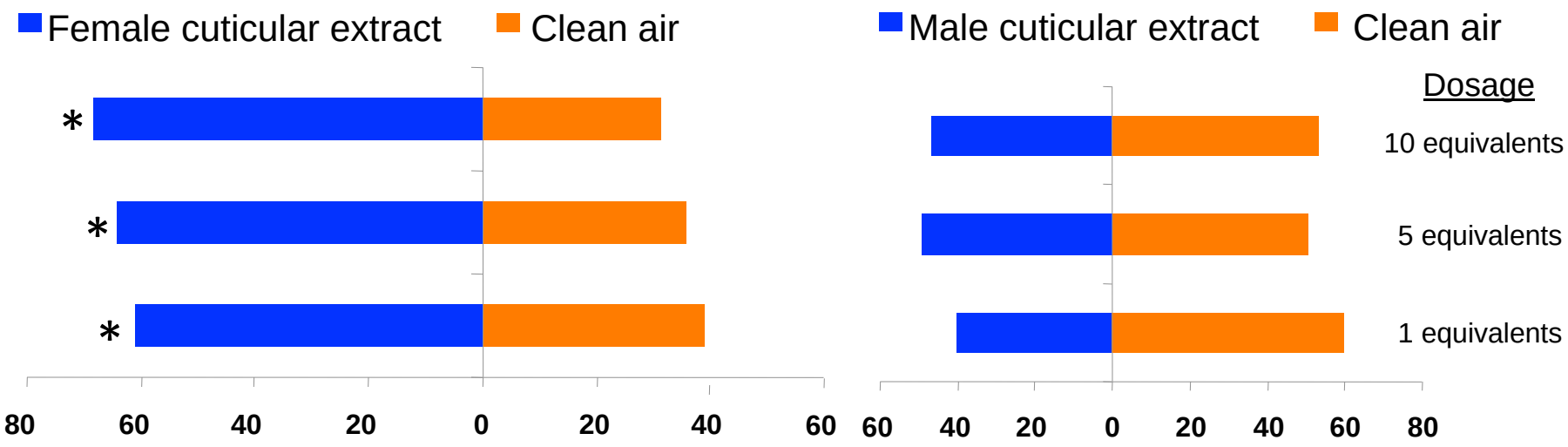
Semiochemicals to manipulate the behavior of ACP

Stelinski

- ACP Sex Pheromones



Female cuticular extract attracts males



Proportion of male *D. citri* responding

Females exhibit no attraction to either male or female extracts in olfactometer assays

In the field, male catch on sticky traps is increased 3.3-4.2 fold on sticky traps with female extract compared with blank controls

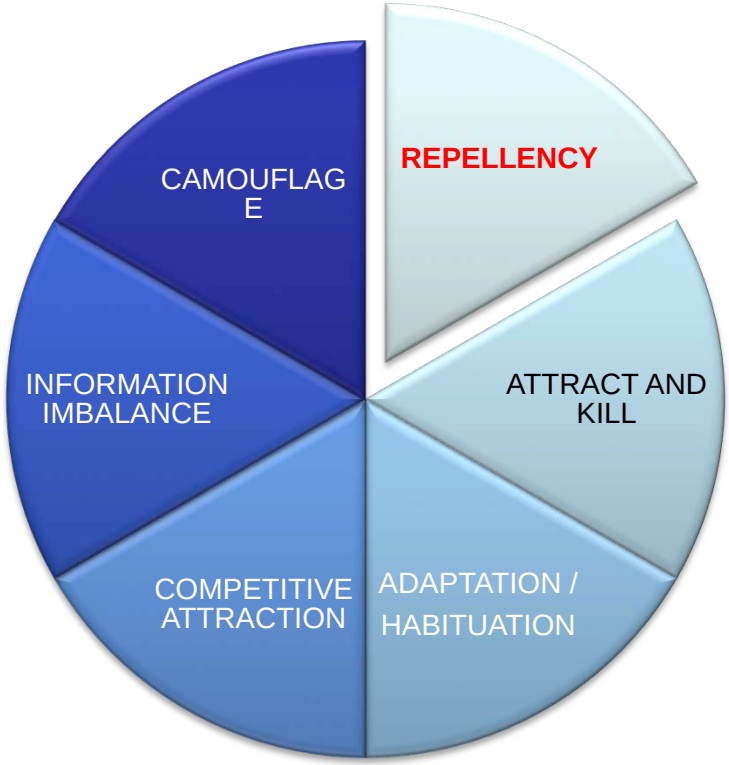
Conclusions on ACP Sex Pheromone

- Females attract males
- Cuticular extracts appear to be source of attractant as seen with other psyllid species
- Not highly potent attractant on sticky traps at 1-10 female equivalent dosages
- Differences exist between male and female cuticular chemistry and we're trying to tease apart which chemicals are the actives

Semiochemicals to Manipulate the Behavior of ACP

- If Strong Sex Pheromones Were Available for ACP, then:
  - Monitoring, even at low densities
  - Control via mating disruption, A&K

ACP Behavior DISRUPTION



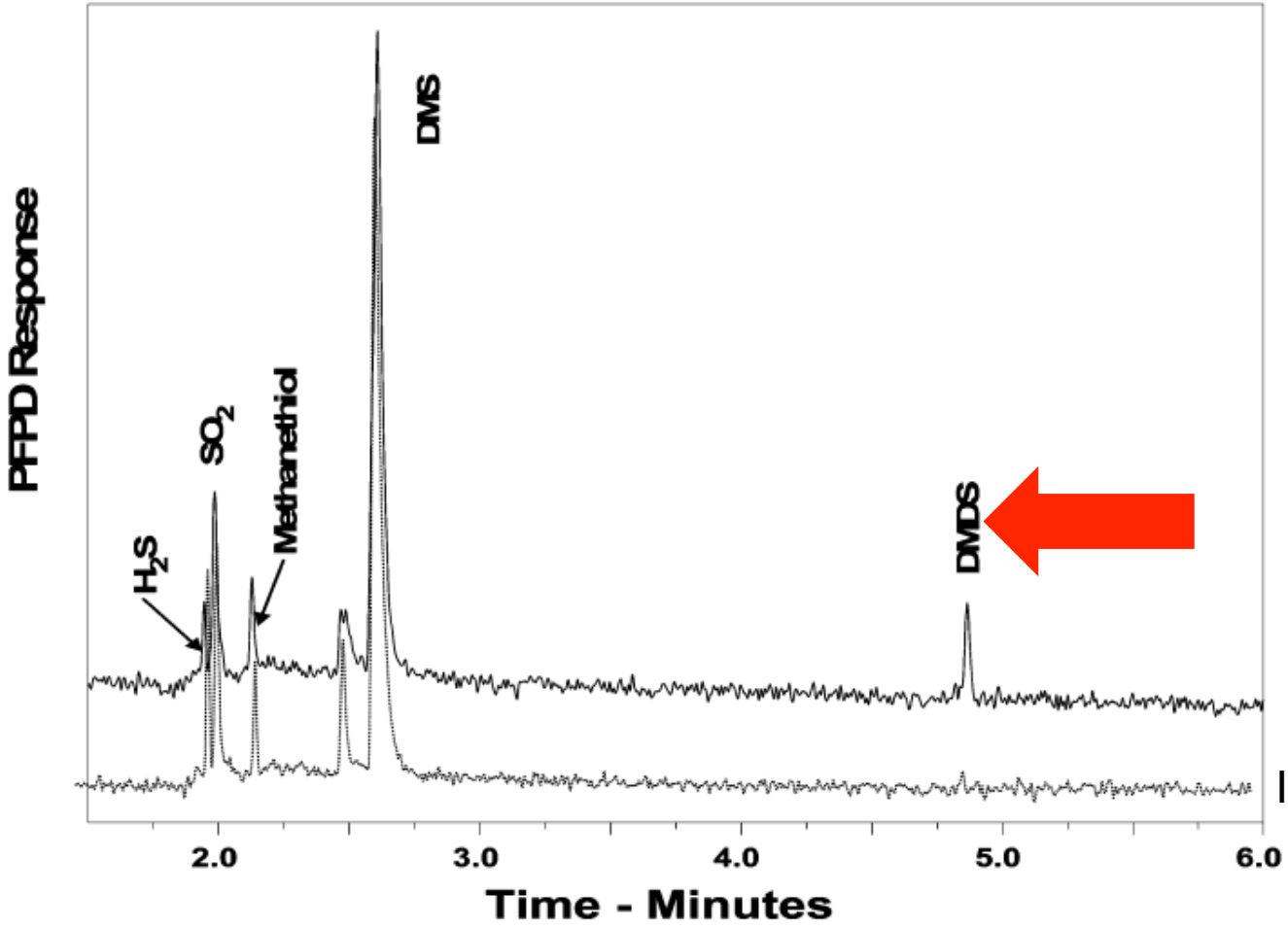
Semiochemicals to Manipulate the Behavior of ACP

Rouseff Stelinski

- Plant Volatiles
  - Repellants: Guava

Headspace From Guava Leaves

Sulfur Volatiles



Crushed leaves

Intact leaves

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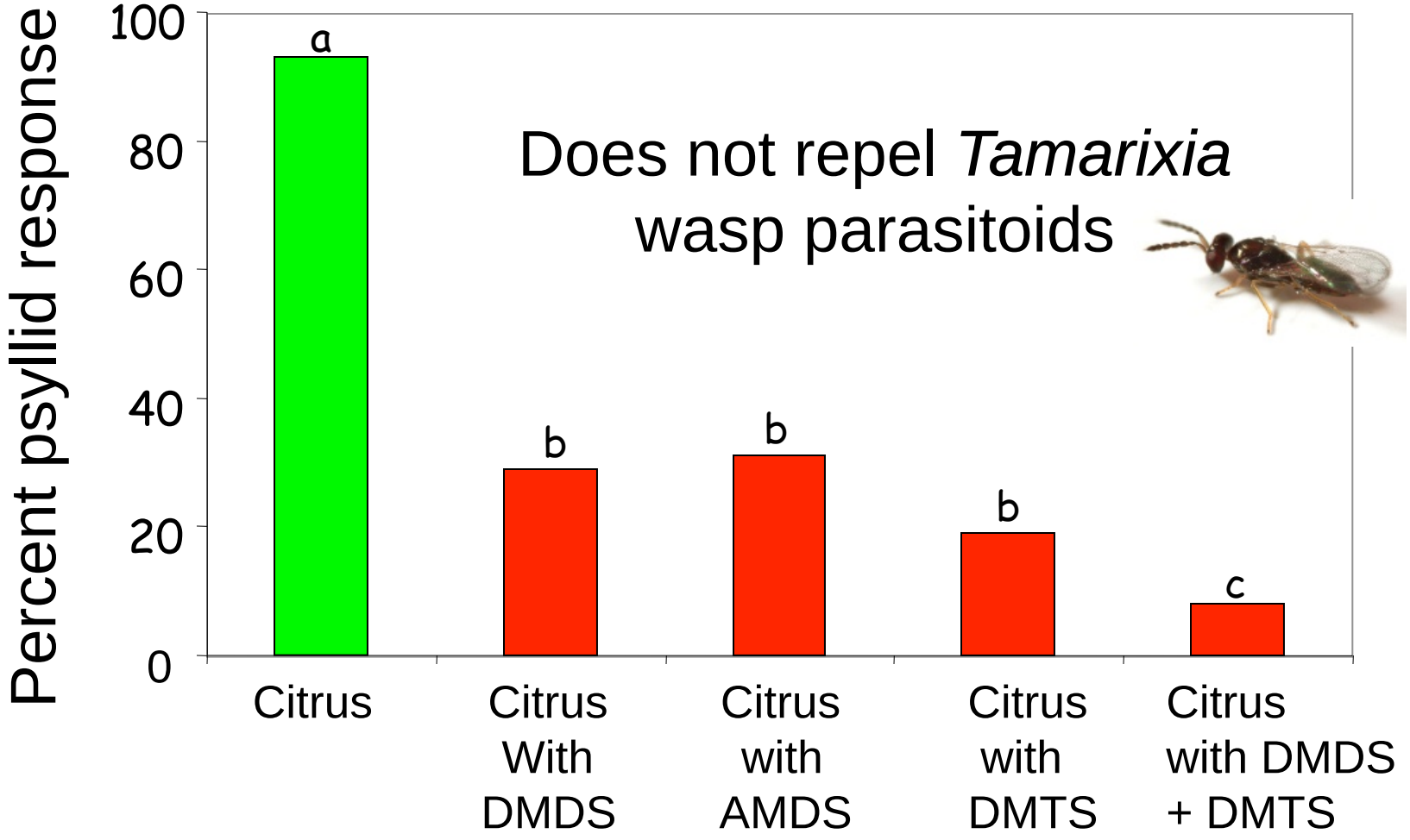
Various Chemical Repellents for ACP Have Been Identified and More are Being Screened



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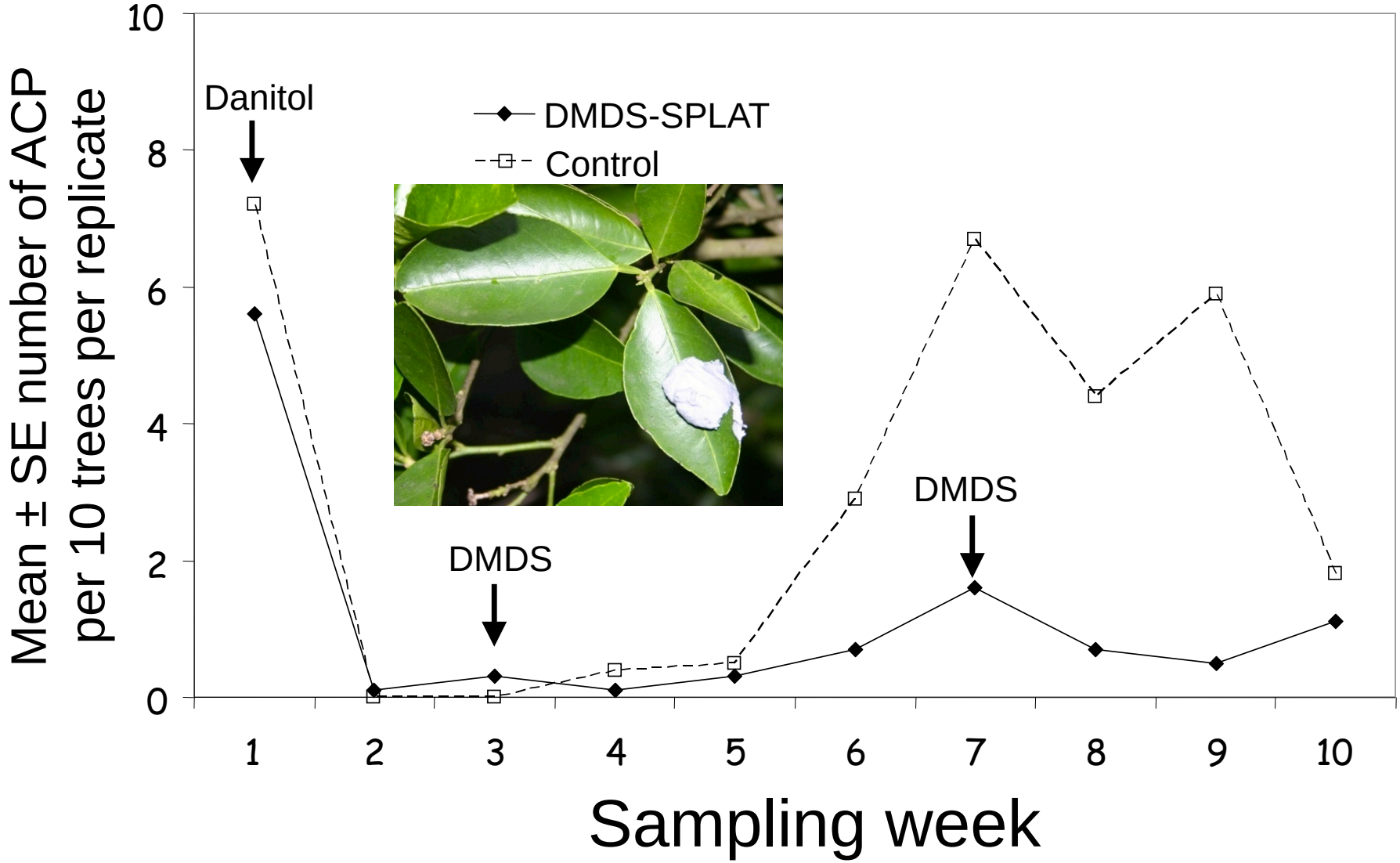
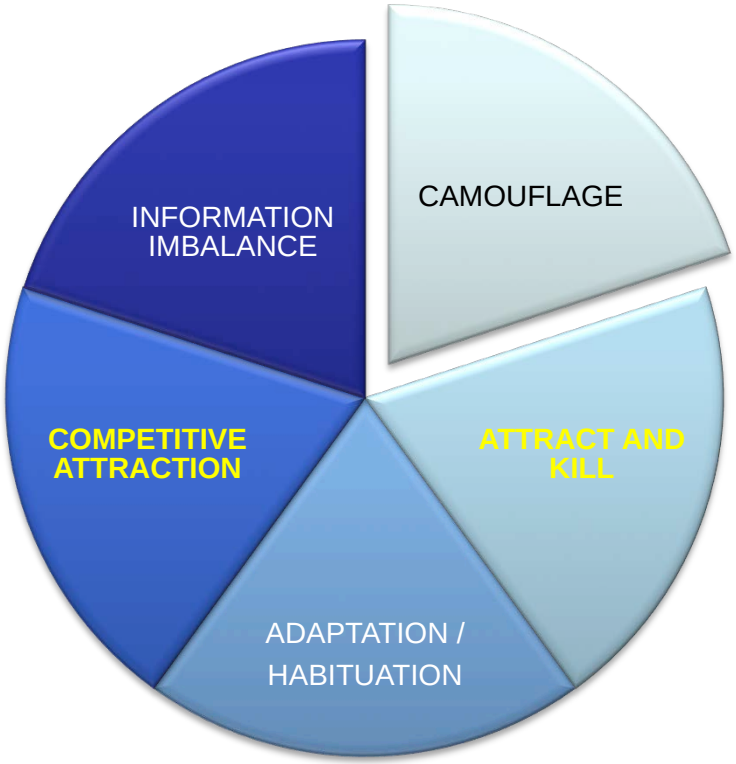
DMDS after Danitol treatment



ACP BEHAVIOR DISRUPTION



Semiochemicals to Manipulate the Behavior of ACP

- Plant Volatiles

Volatiles From Citrus Shoots as Attractants to ACP

Hall, Lapointe, Patt, Robbins, Setamou, Stelinski

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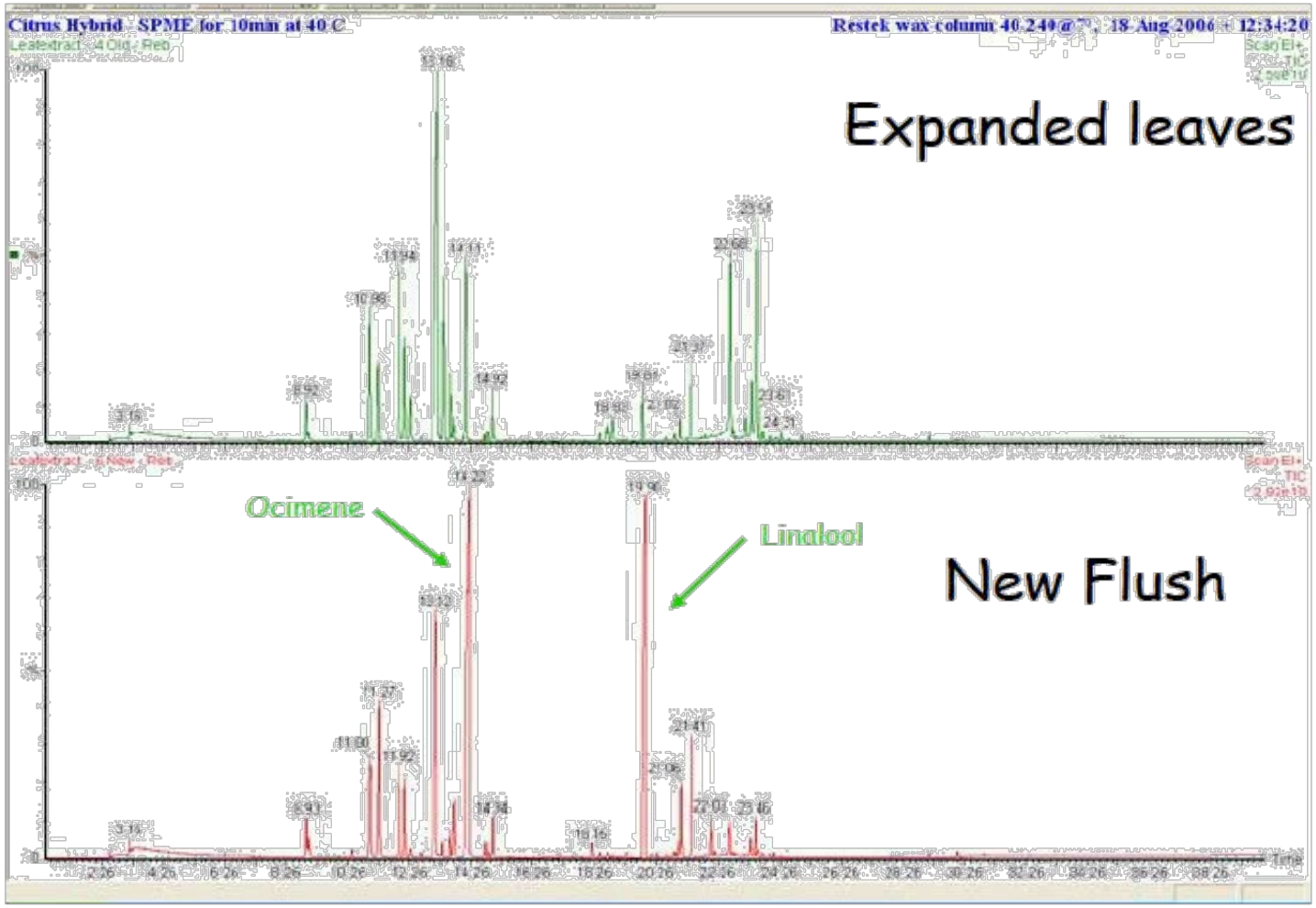
Chemical analysis of citrus volatiles: there are quantitative differences in volatiles produced between expanded leaves and new flush

Stelinski



Photos: M. Setamou

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Volatiles From Citrus Shoots as Attractants to ACP

Hall

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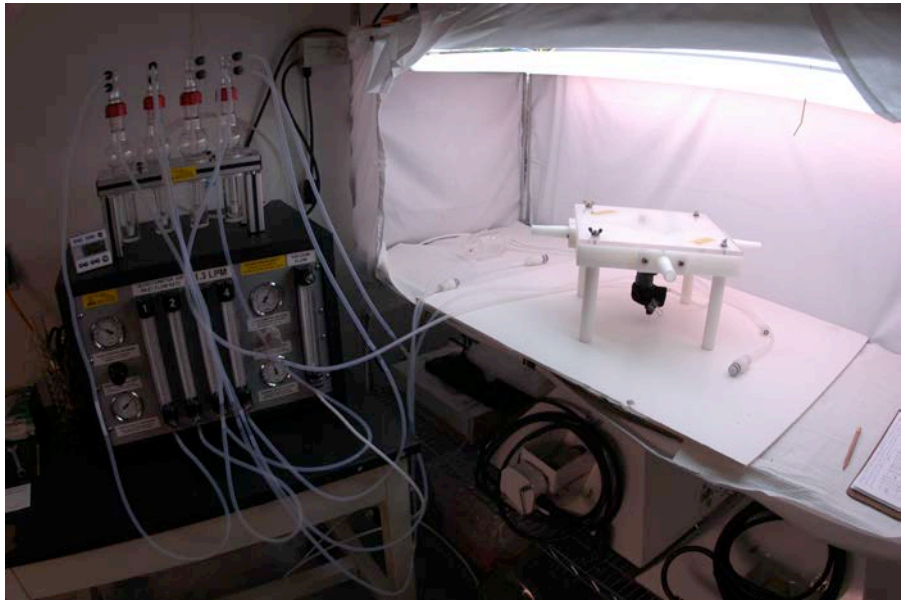
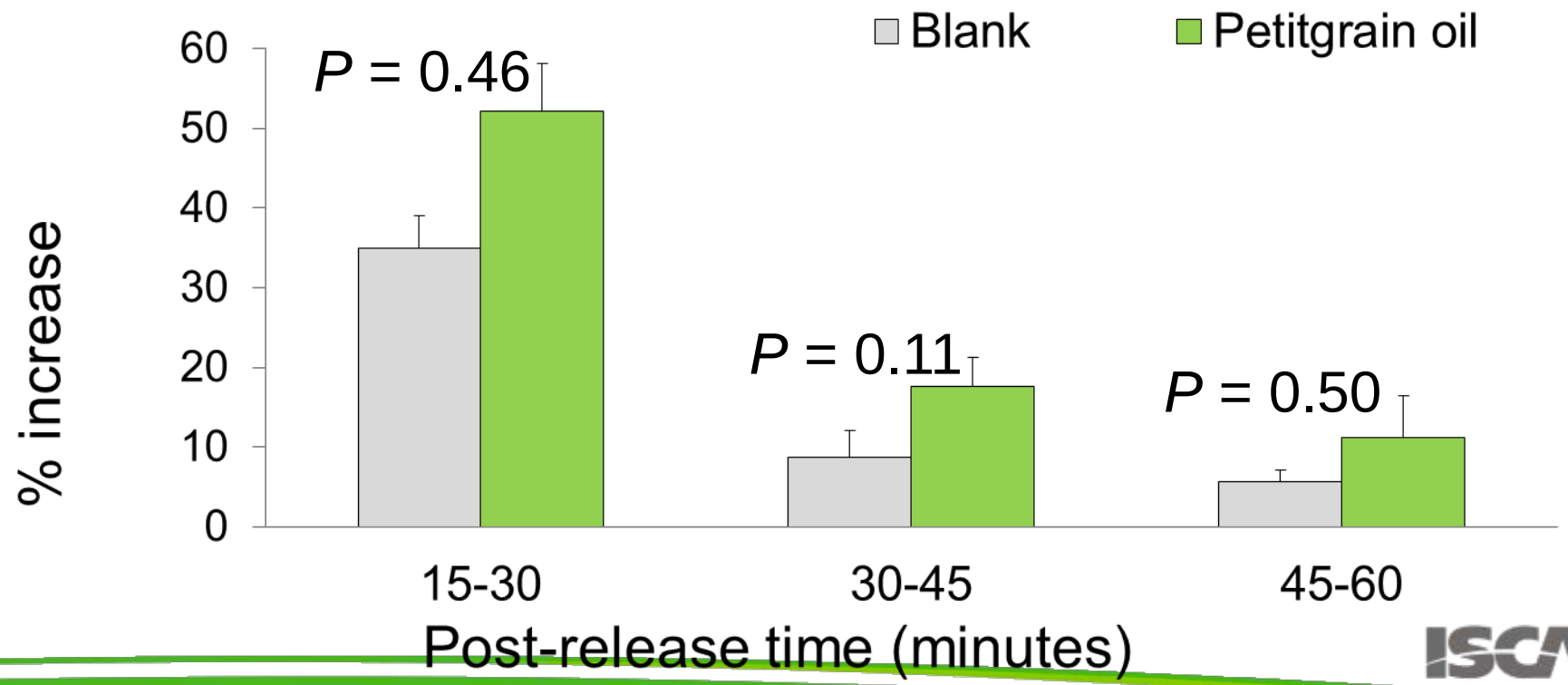
Hall



Volatiles From Citrus Shoots as Attractants to ACP

Patt

Effect of Petitgrain Oil on ACP Capture Rate in Greenhouse Tests



t-test, $P \leq 0.05$, n = 5 replicates

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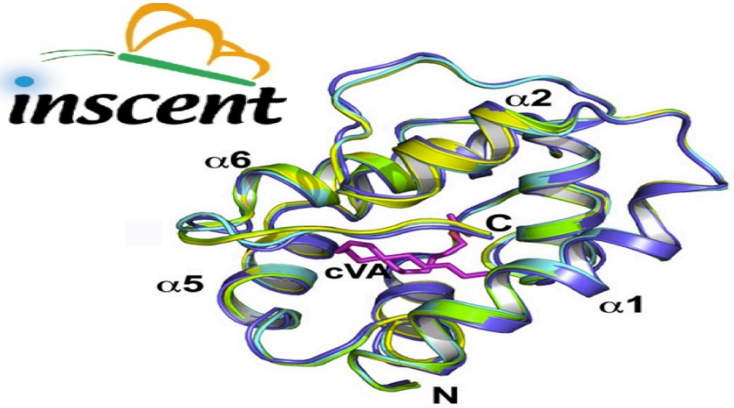
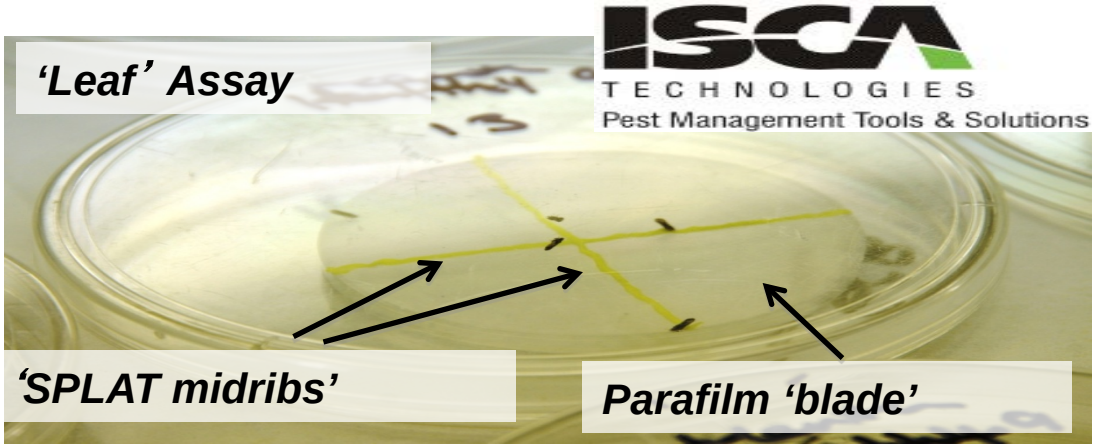
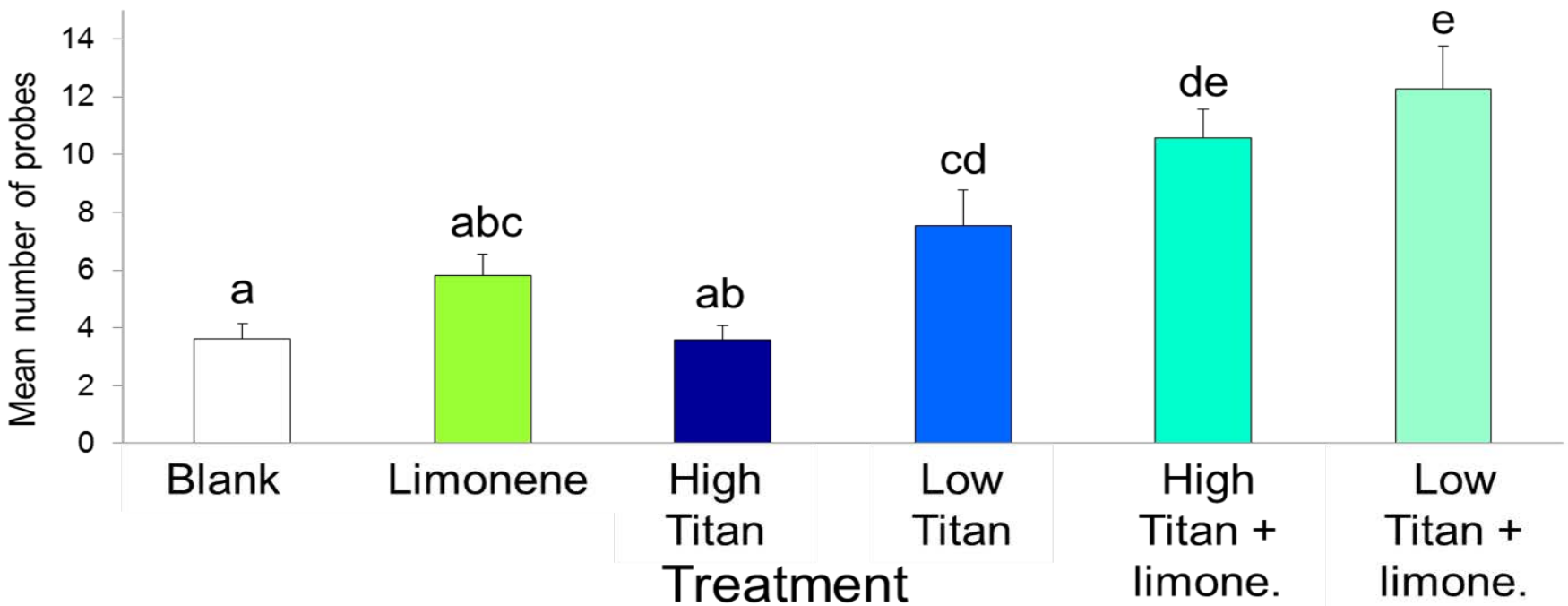


Olfactory binding protein

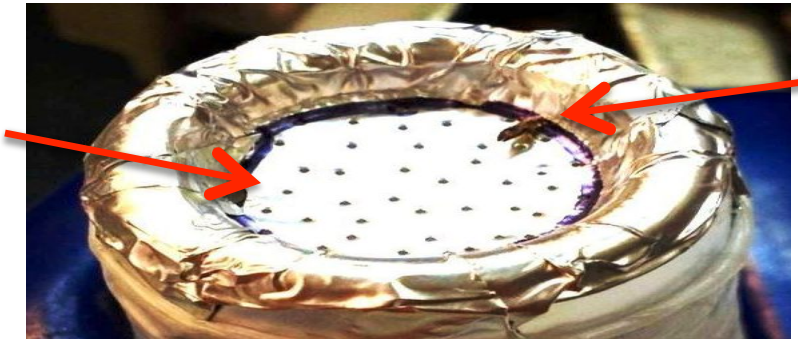


Response to Novel Scent Mimics--Probing



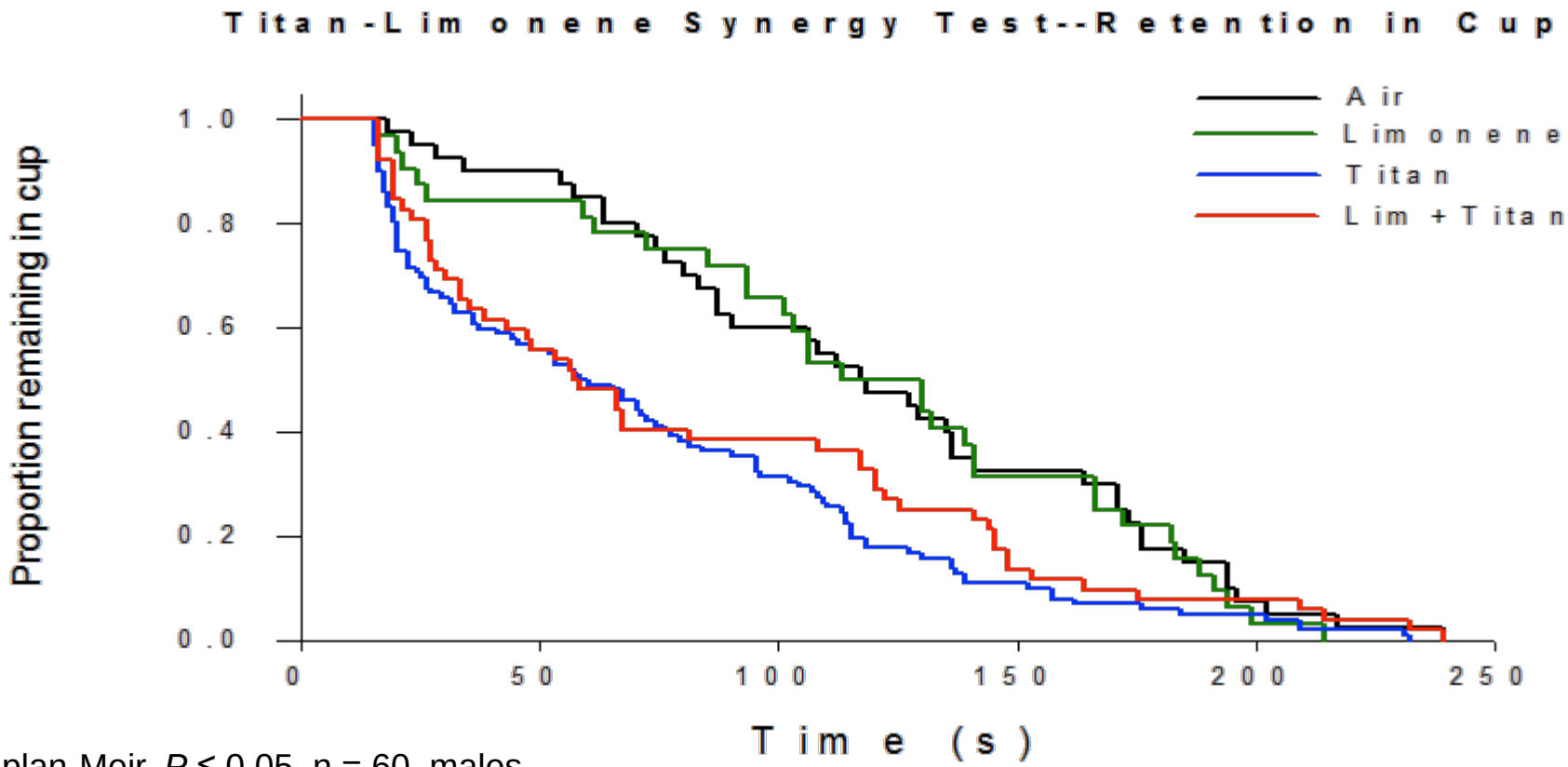
Proc. Mixed SAS, $P \leq 0.05$, n = 30, mixed sexes

Perforated Foil Cup



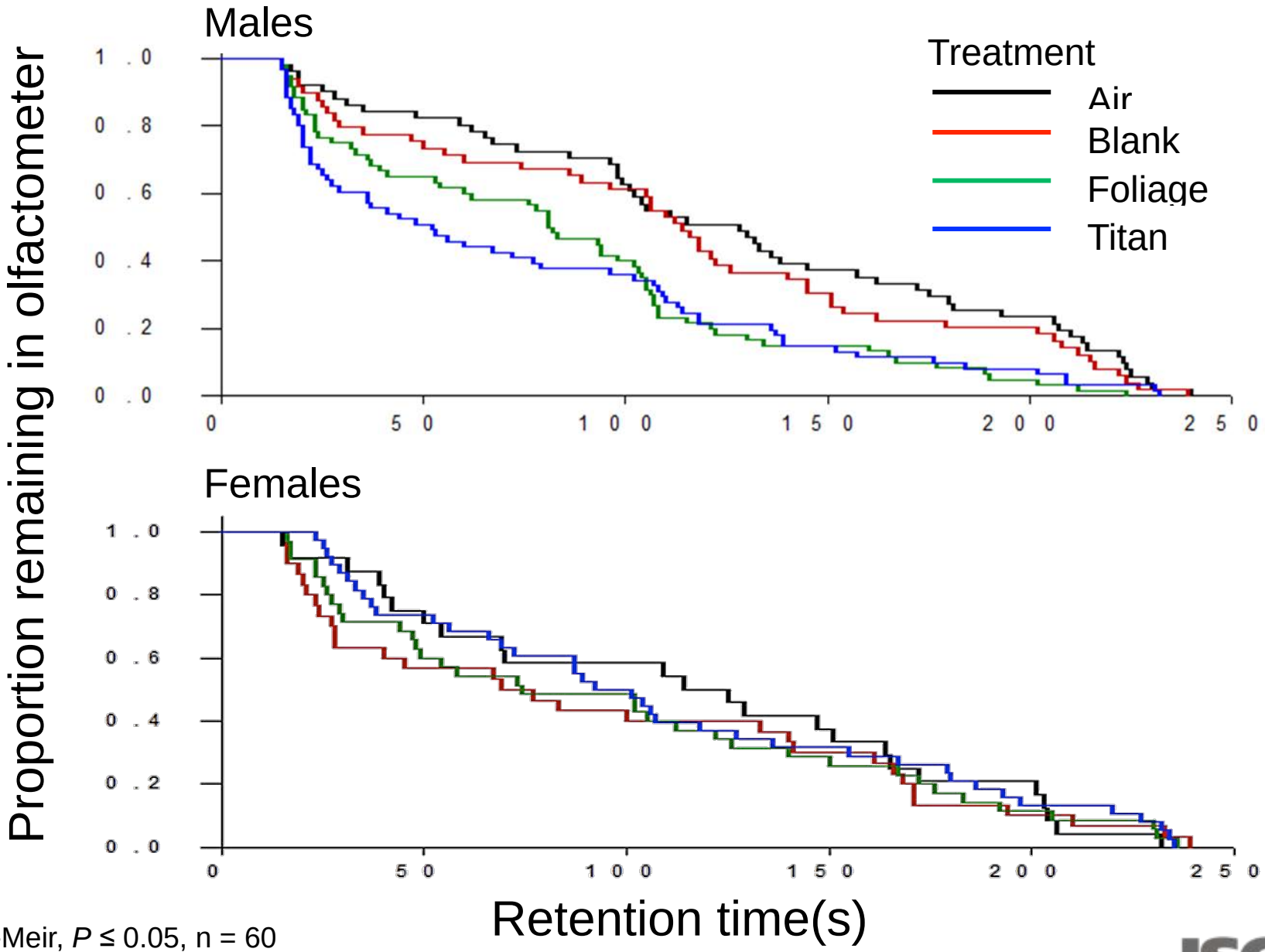
ACP

Response to Novel Scent Mimics--Olfaction



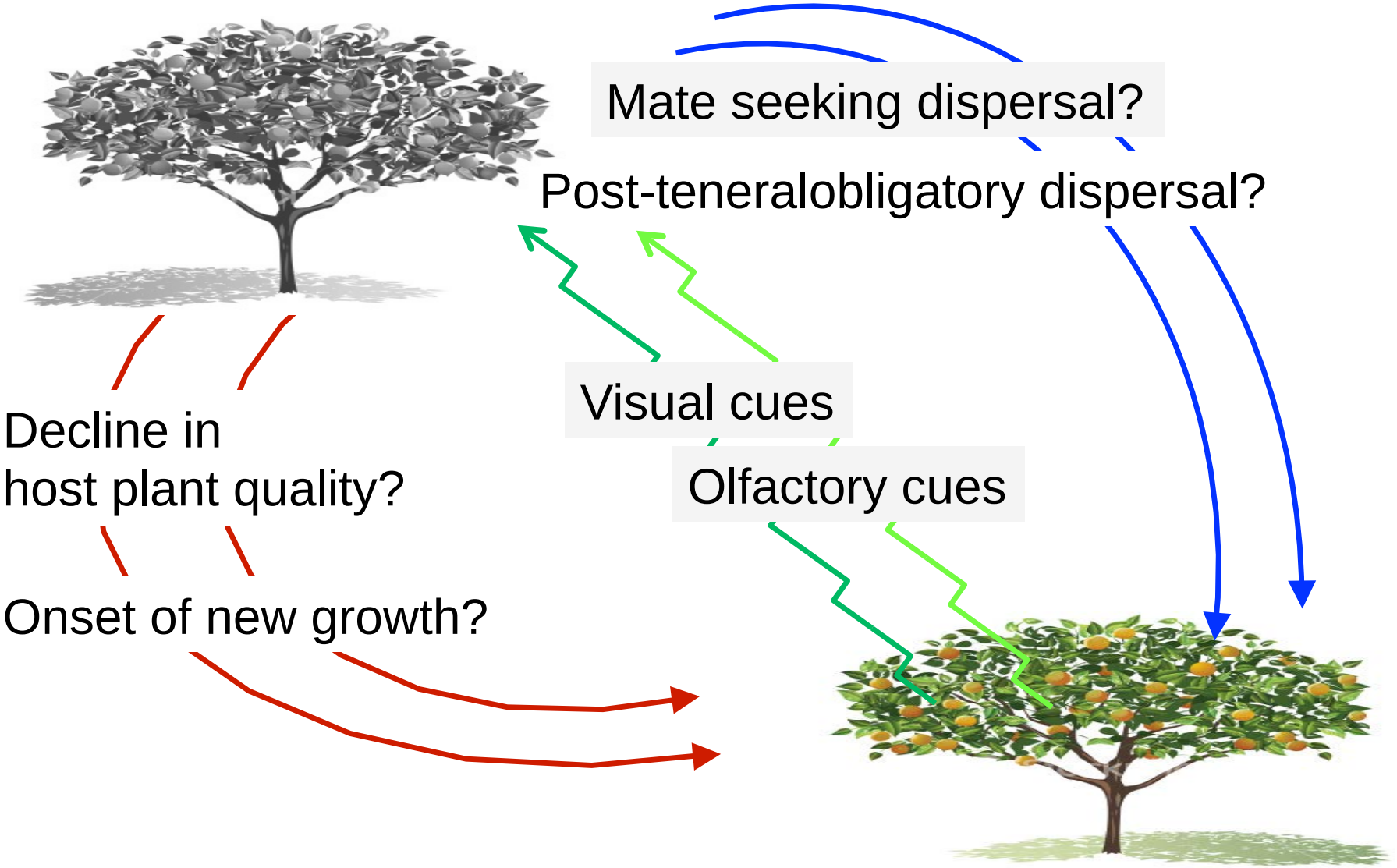
Kaplan-Meir, $P \leq 0.05$, n = 60, males

Olfactometer Test: Titan v. *Murraya* foliage



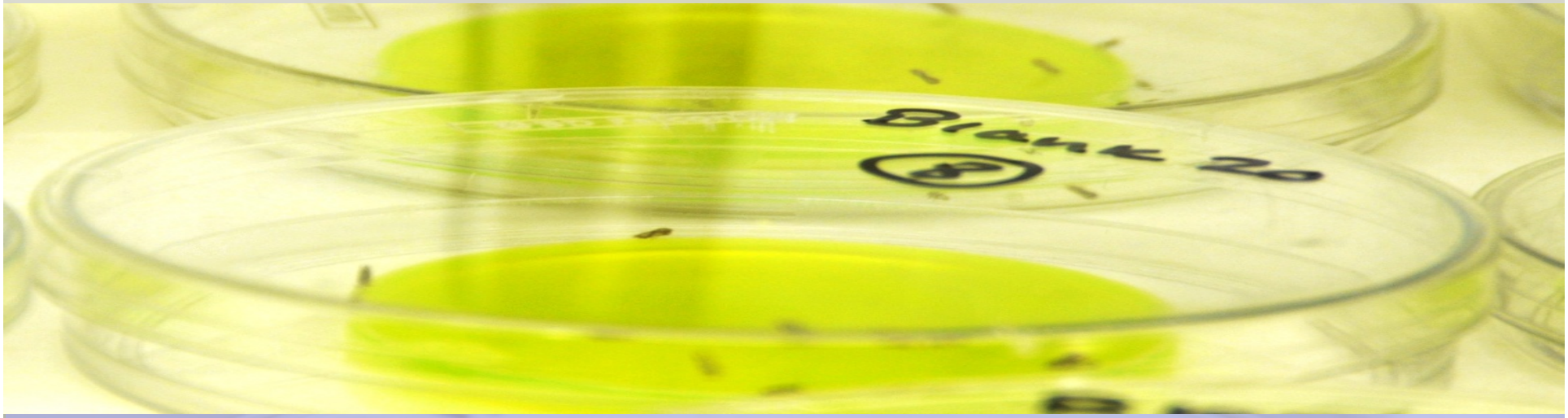
Kaplan-Meir, $P \leq 0.05$, n = 60

Potential Dispersal Triggers in ACP

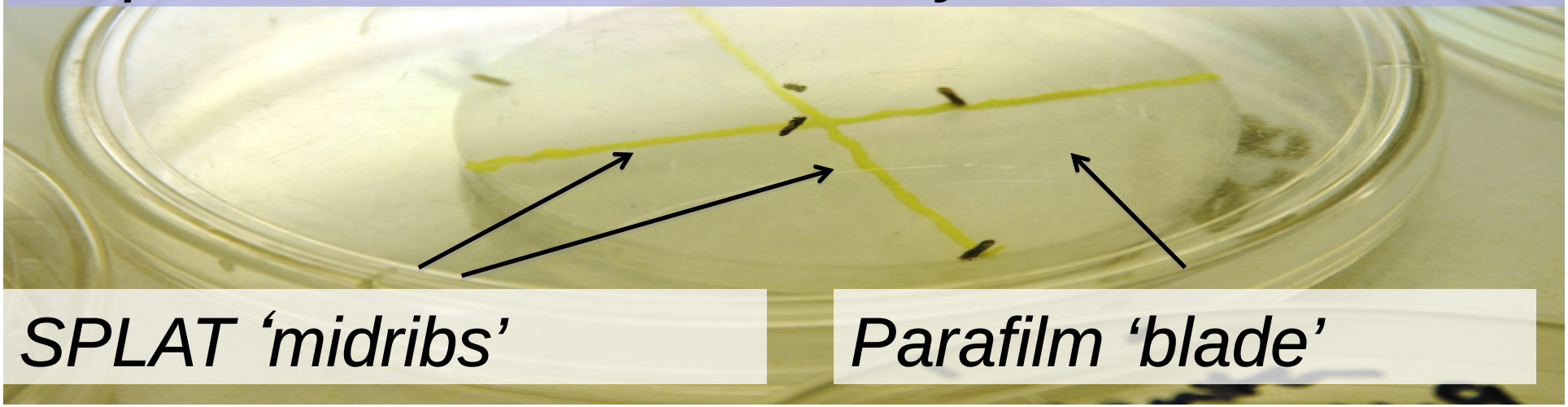


Innate v. Conditioned Responses

Step 1--Conditioning Treatment: Sucrose Solution Plus Flavorant in Membrane Feeder



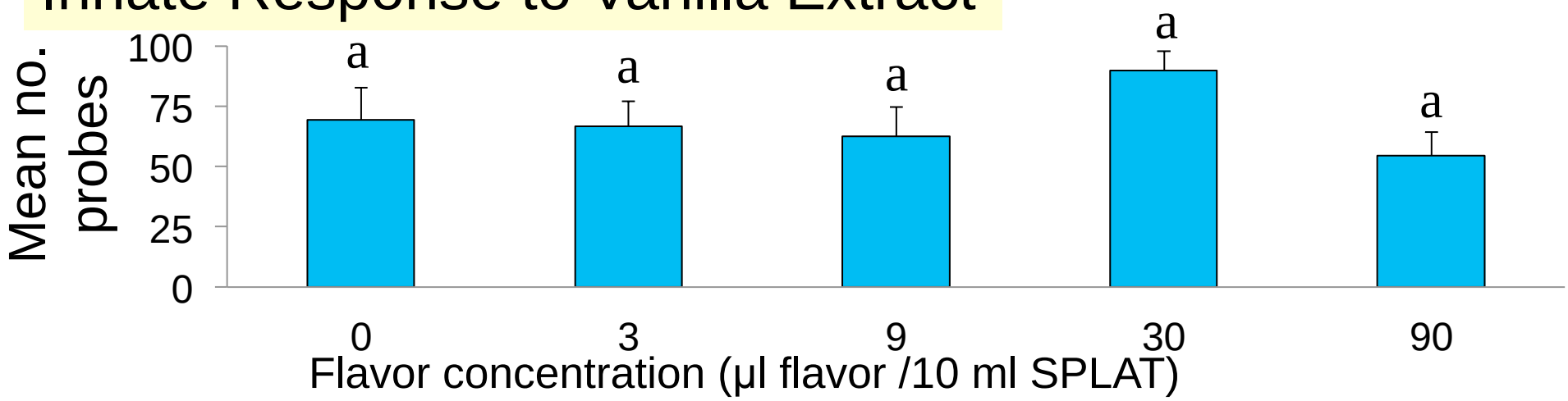
Step 2—Test with 'Leaf' Assay



SPLAT 'midribs'

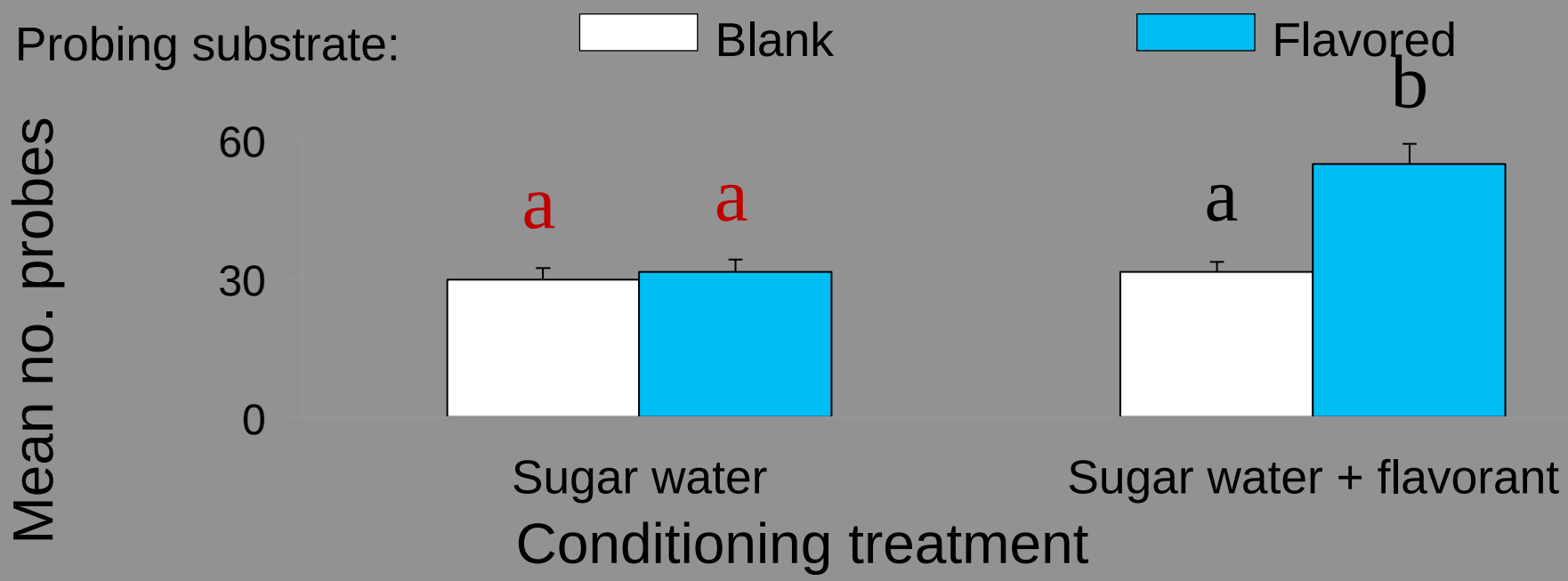
Parafilm 'blade'

Innate Response to Vanilla Extract



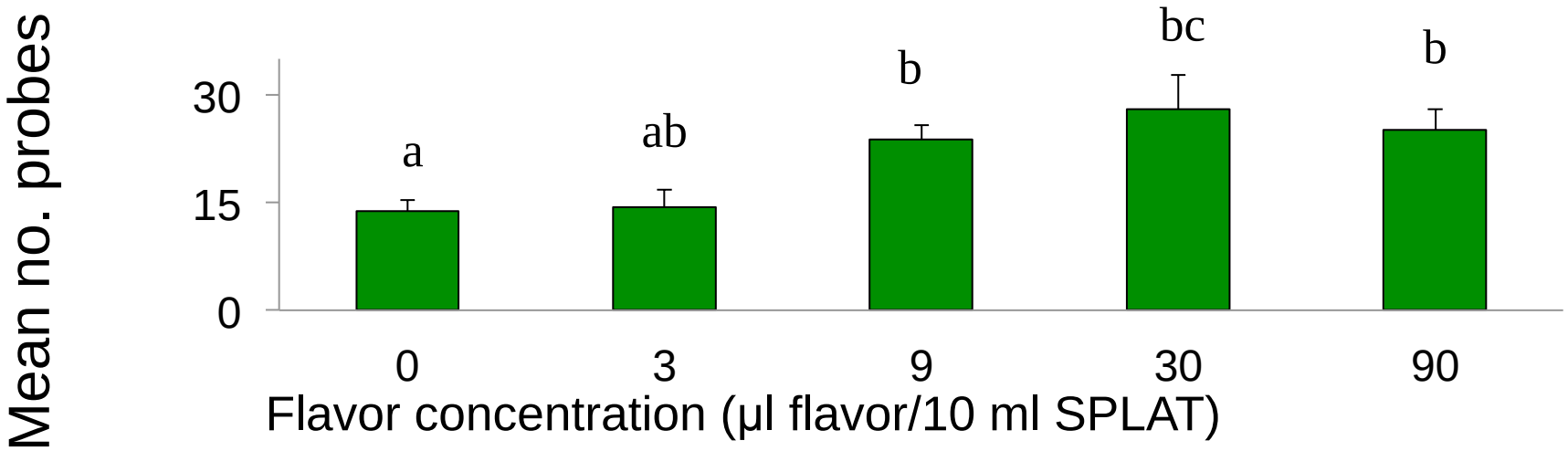
Proc. Mixed SAS, $P \leq 0.05$, n = 30, mixed sexes

Conditioned Response to Vanilla Extract



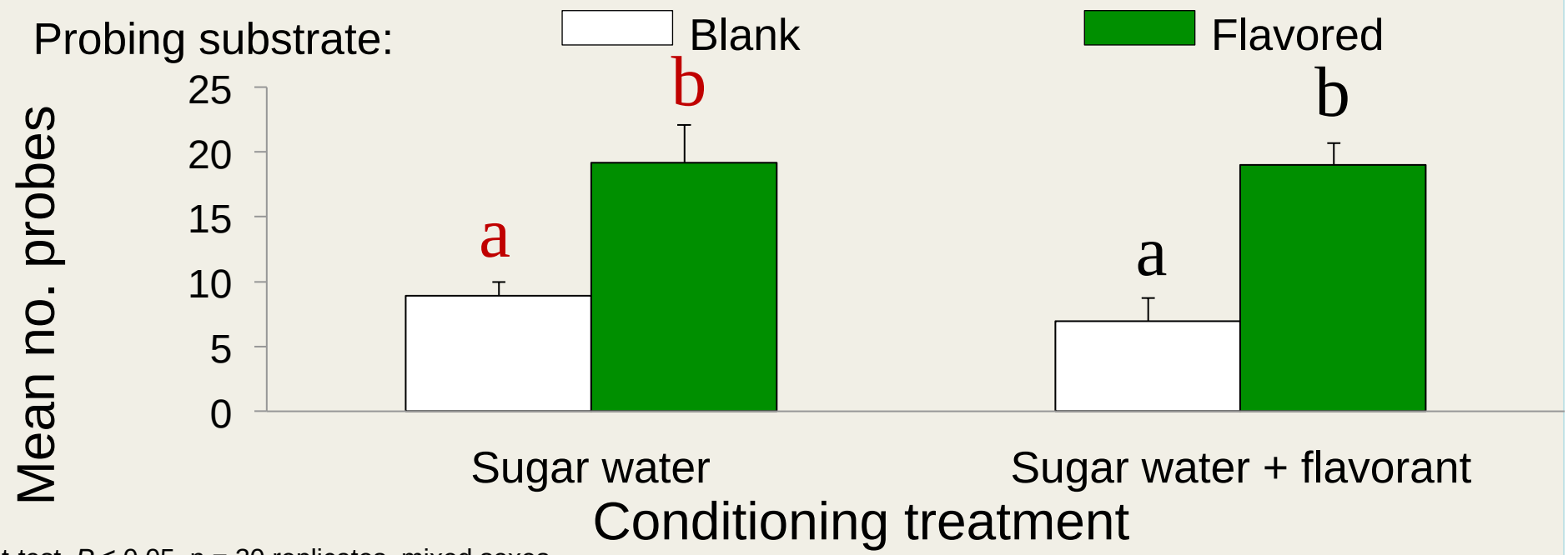
t-test, $P \leq 0.05$, n = 30 replicates, mixed sexes

Innate Response to Lemon Extract



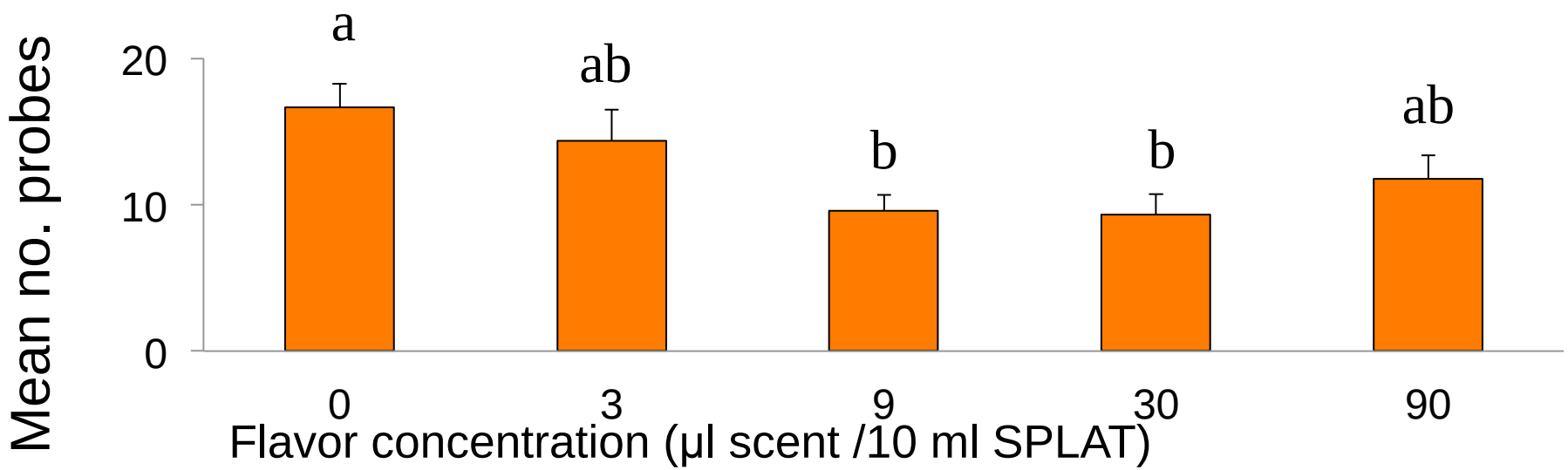
Proc. Mixed SAS, P ≤ 0.05, n = 30, mixed sexes

Conditioned Response to Lemon Extract



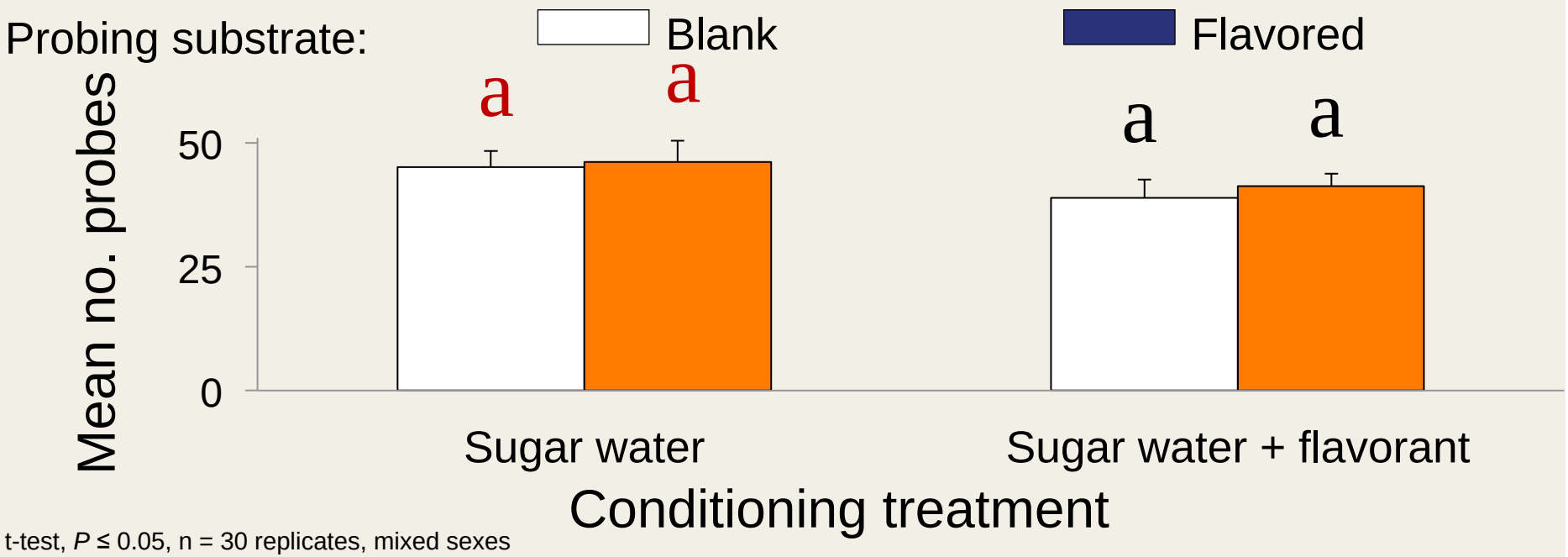
t-test, *P* ≤ 0.05, n = 30 replicates, mixed sexes

Innate Response to Banana Extract



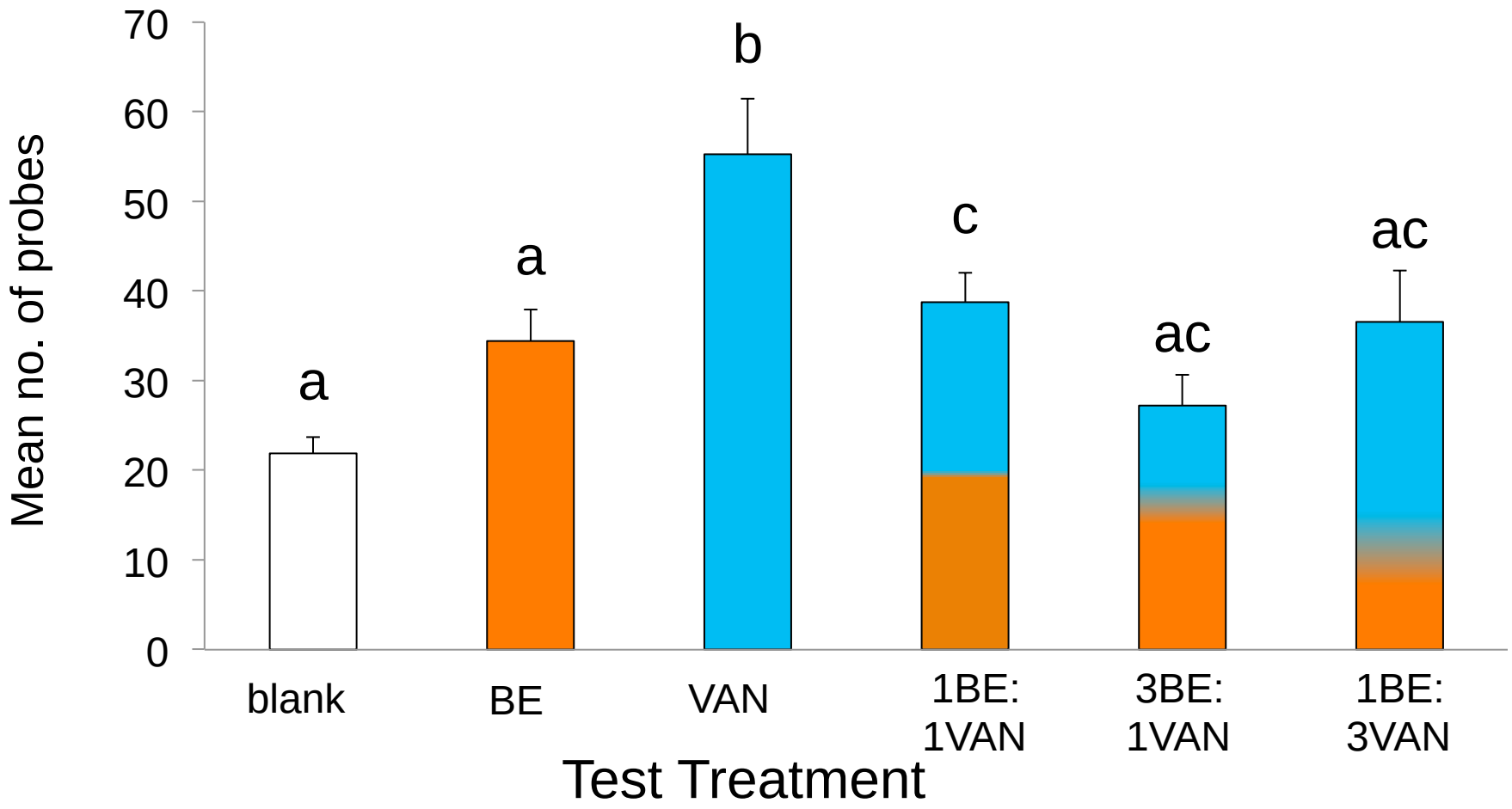
Proc. Mixed SAS, P ≤ 0.05, n = 30, mixed sexes

Conditioned Response to Banana Extract



t-test, *P* ≤ 0.05, n = 30 replicates, mixed sexes

Response to Banana Extract (BE), Vanilla extract (VAN), and Mixtures Following Conditioning to Vanilla Extract



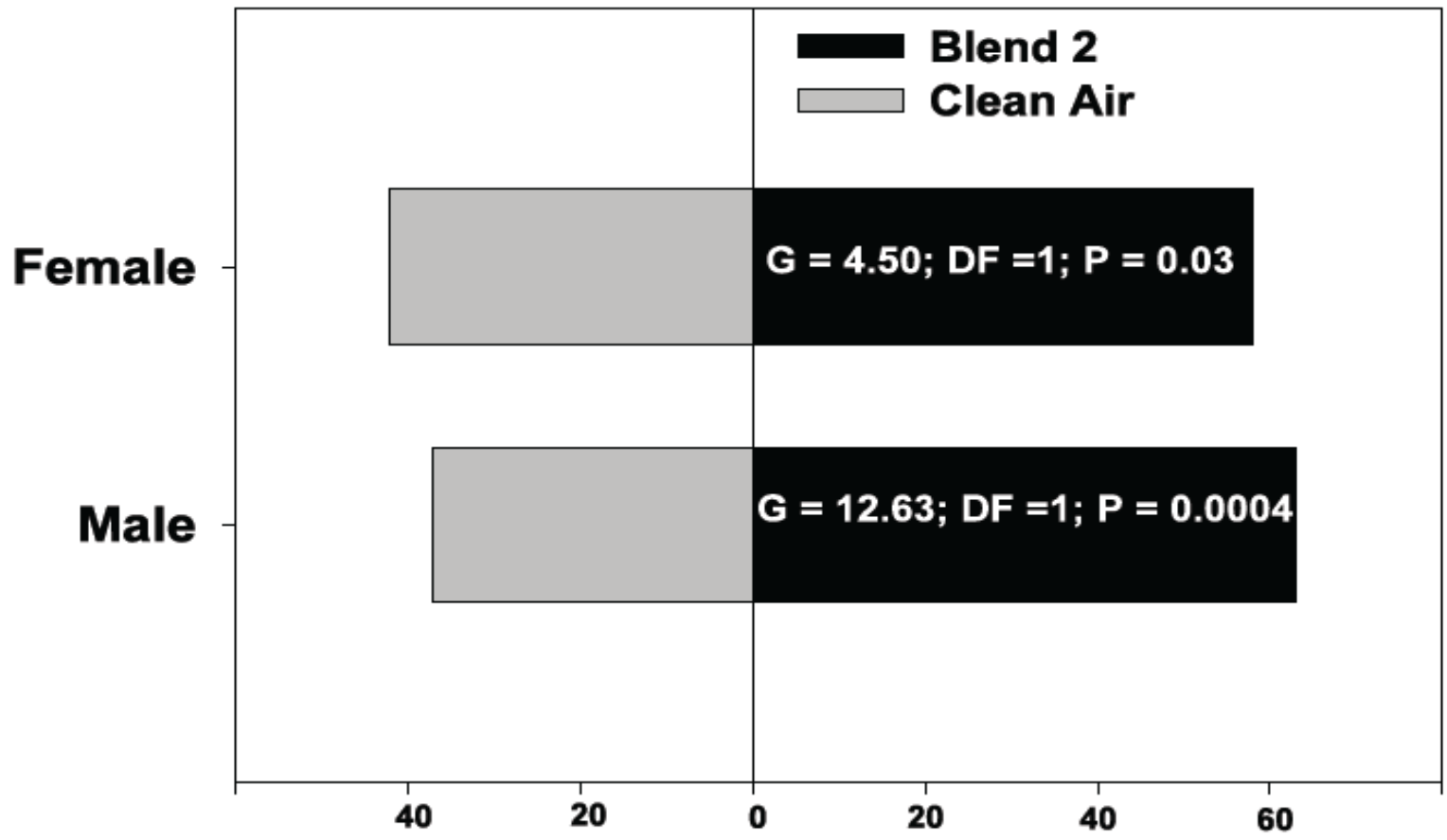
Proc. Mixed SAS, P ≤ 0.05, n = 30, mixed sexes

Volatiles From Citrus Shoots as Attractants to ACP

Setamou

Y-tube olfactometer used to evaluate odor preference of *D. citri* adults

Response of *D. citri* to odors of a synthetic blend in Y-tube olfactometer



Reponse (%) of *D. citri* adults

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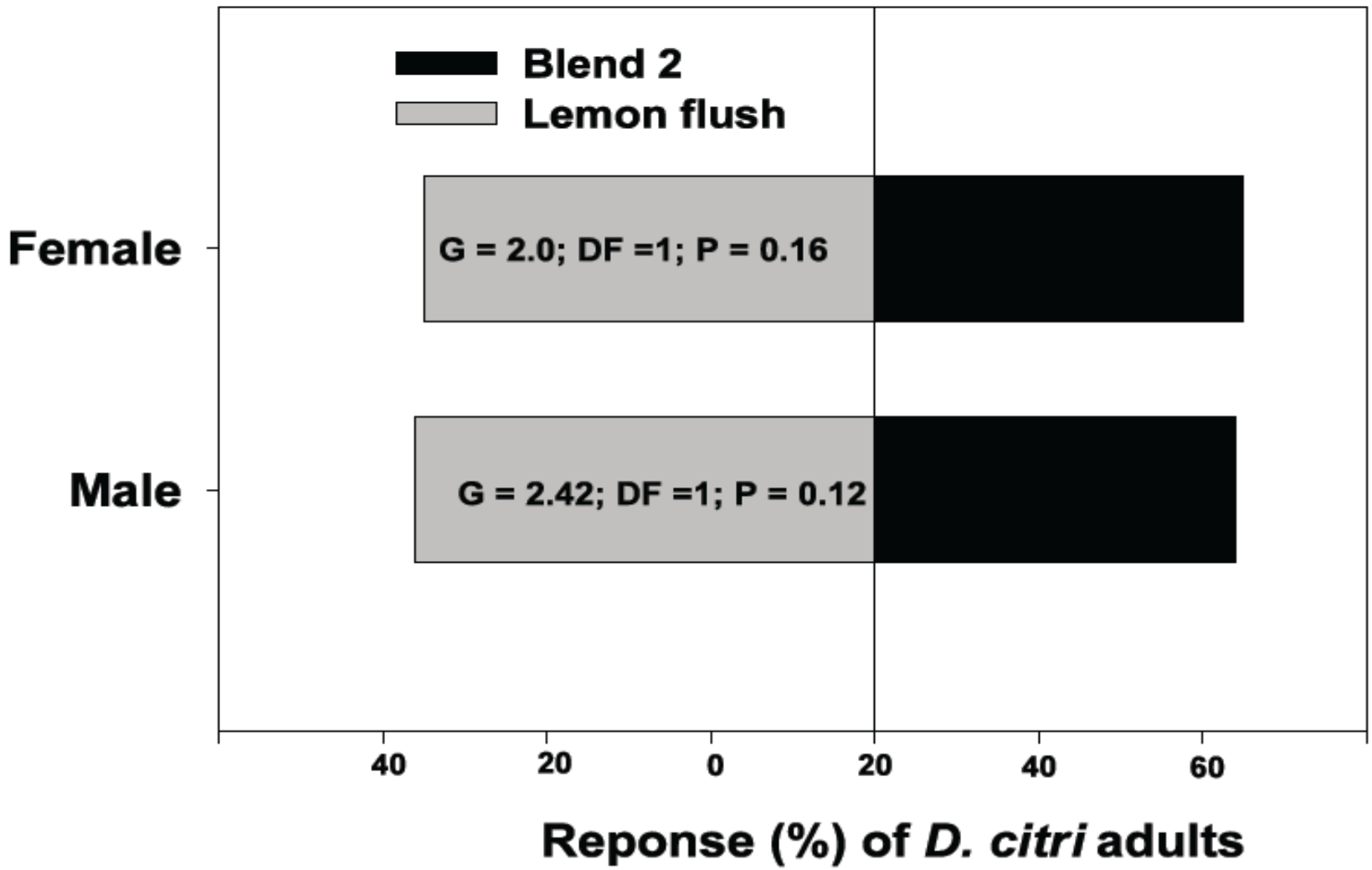
Response of *D. citri* to odors of a synthetic blend and lemon flush shoots in Y-tube olfactometer



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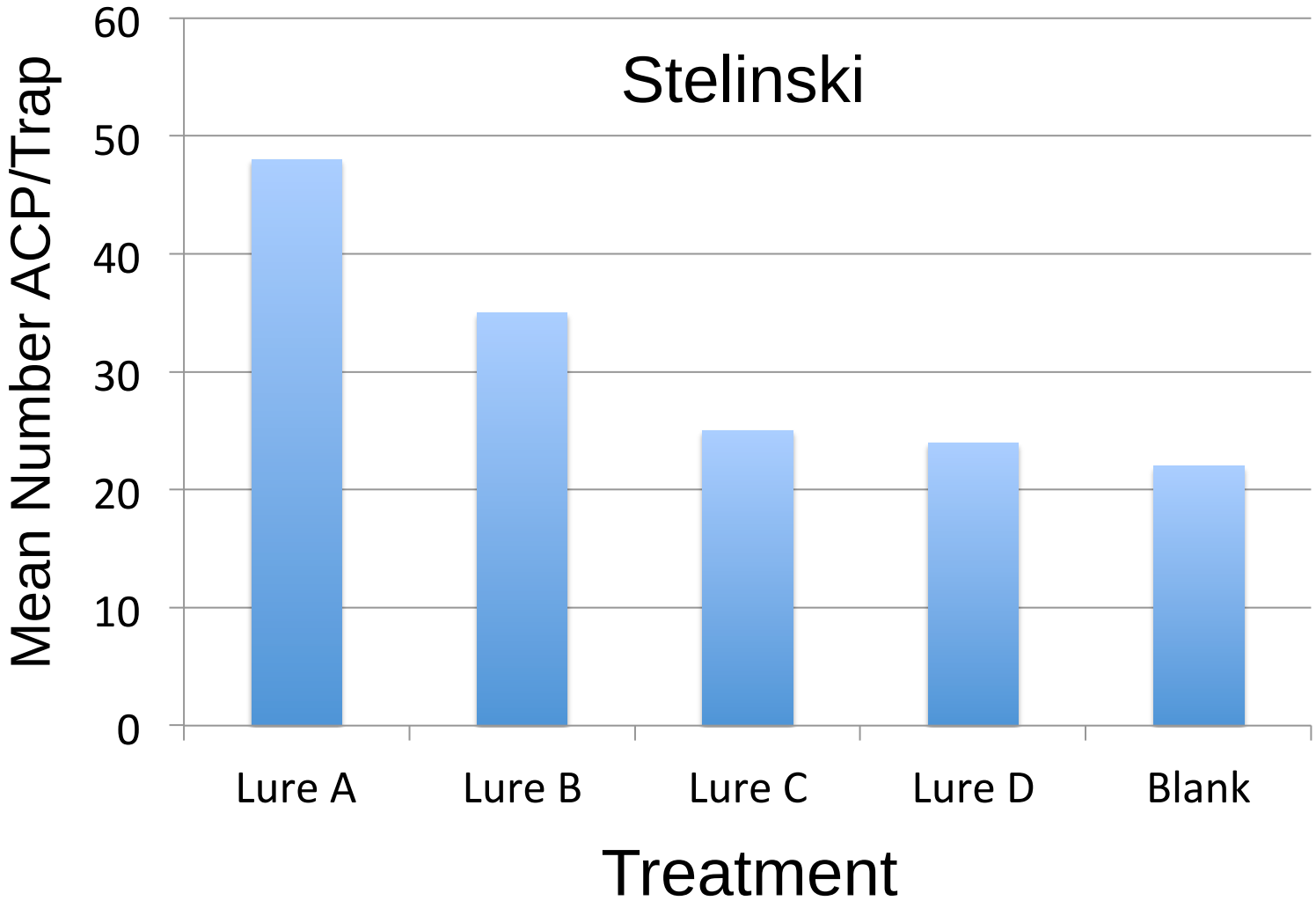
FIELD TRIALS

- Plant Volatiles – Field Trials

Stelinski

We now have plant volatile lures that significantly increase catch of psyllids on traps



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It looks great: we have good candidate plant volatile attractants

But we don’t have a plant attractant attractive enough to formulate in SPLAT and bring to the field yet.

We are hoping for an **Attract and Kill** formulation, or to integrate it in a **Push and Pull** system.

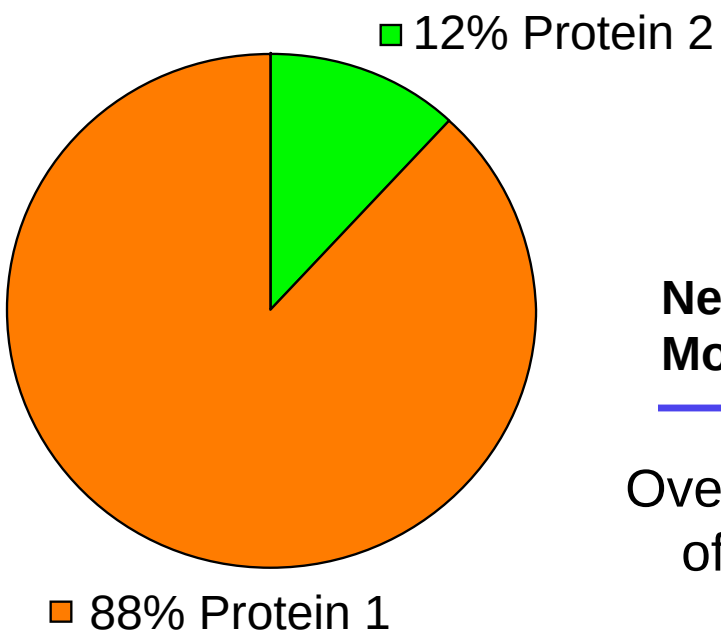
Semiochemicals to Manipulate the Behavior of ACP

- Mating Pheromones
- Plant Volatiles
  - New Shoots
  - Damage
    - ACP
      - Natural Enemies
    - HLB

Stelinski

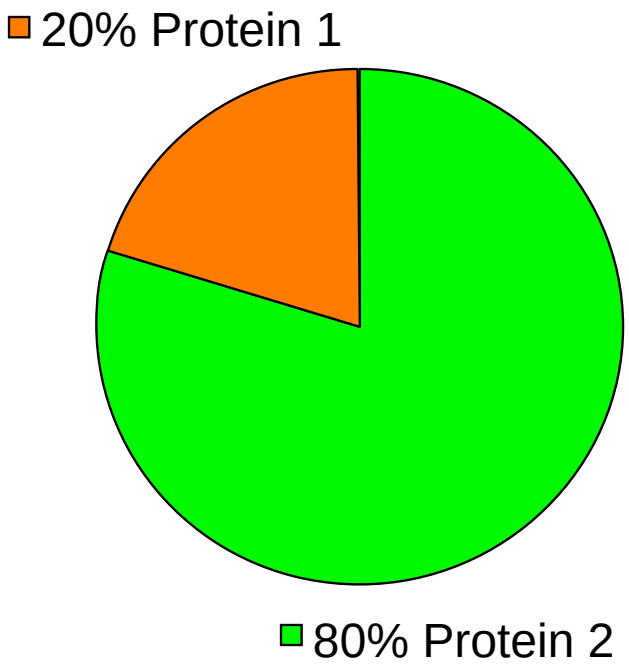
ACP movement is biased toward managed groves when unmanaged and managed groves are adjacent

Percentage of captured ACP marked with protein markers



Net ACP Movement

Over the course of two days



Unmanaged Grove marked with Protein 1

Managed Grove marked with Protein 2

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Chemical Ecology of the Interactions ACP/HLB/CITRUS/Beneficials

Stelinski

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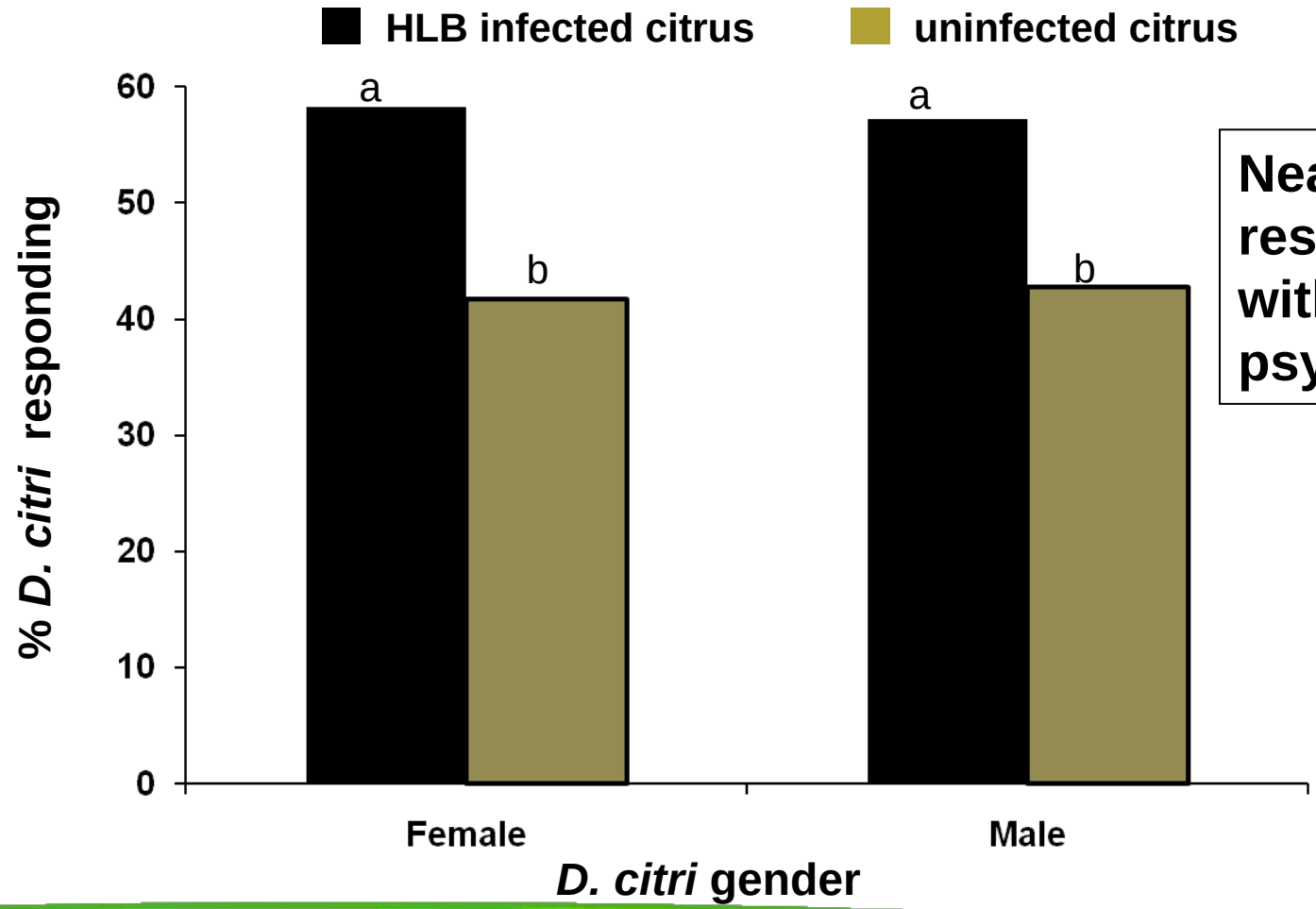
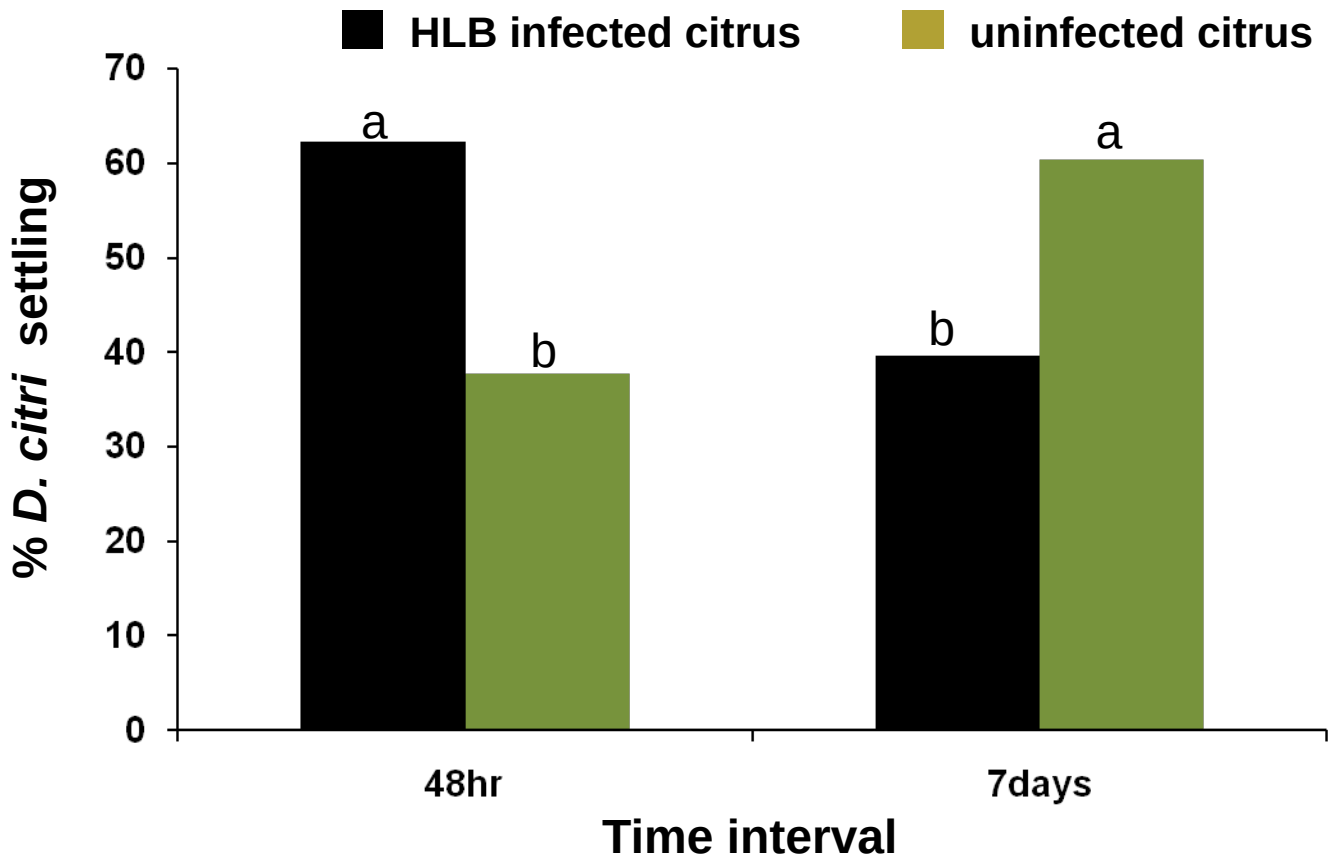
Response of ACP to odors emitted from HLB infected and uninfected citrus in an olfactometer.



Nearly identical results obtained with Las-infected psyllids.

Settling preference of ACP on HLB infected and uninfected citrus plants.



Nearly identical results obtained under dark conditions and with Las infected.

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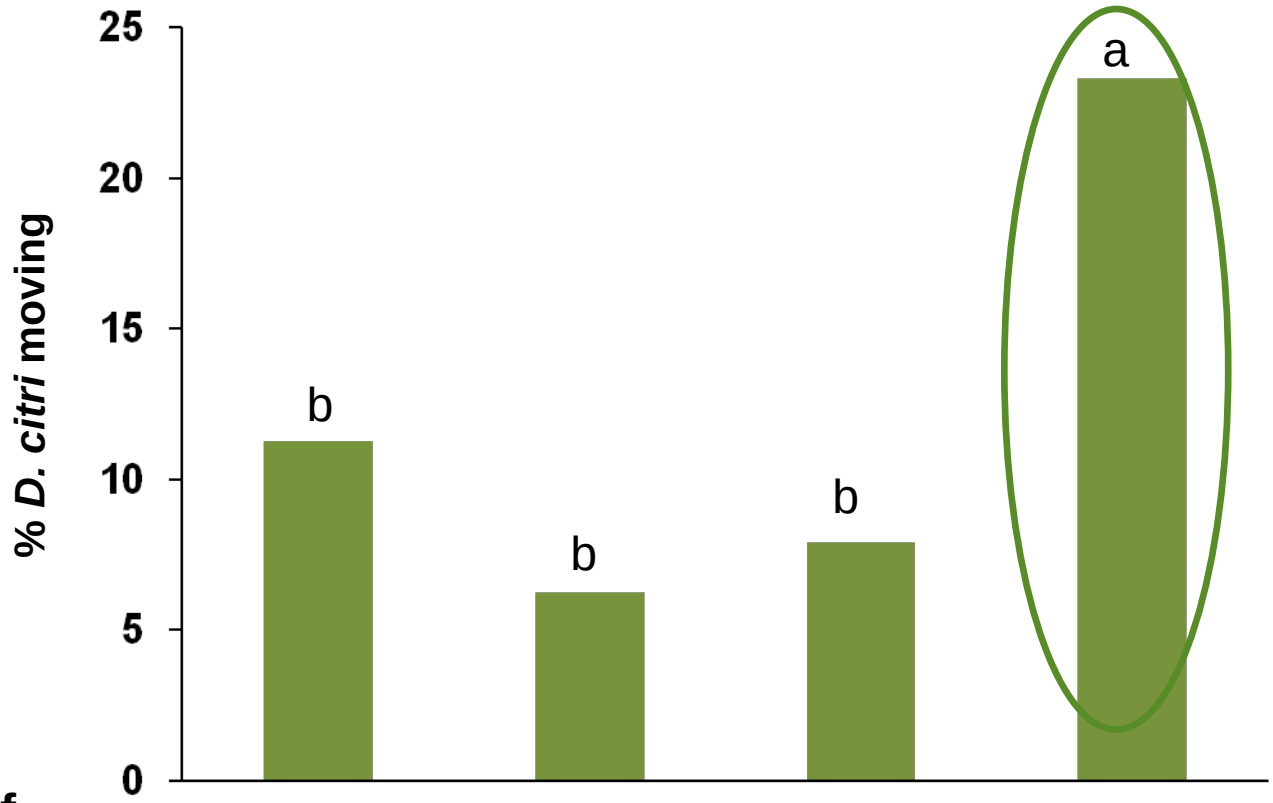
Choice Assays Between Infected and Uninfected Plants

Movement of *A*CP from HLB Infected to Uninfected Citrus Plants



Status of release plant :	**Uninfected**	**Uninfected**	**Infected**	**Infected**
Status of second plant :	**Uninfected**	**Infected**	**Infected**	**Uninfected**

Headspace Volatile Collections

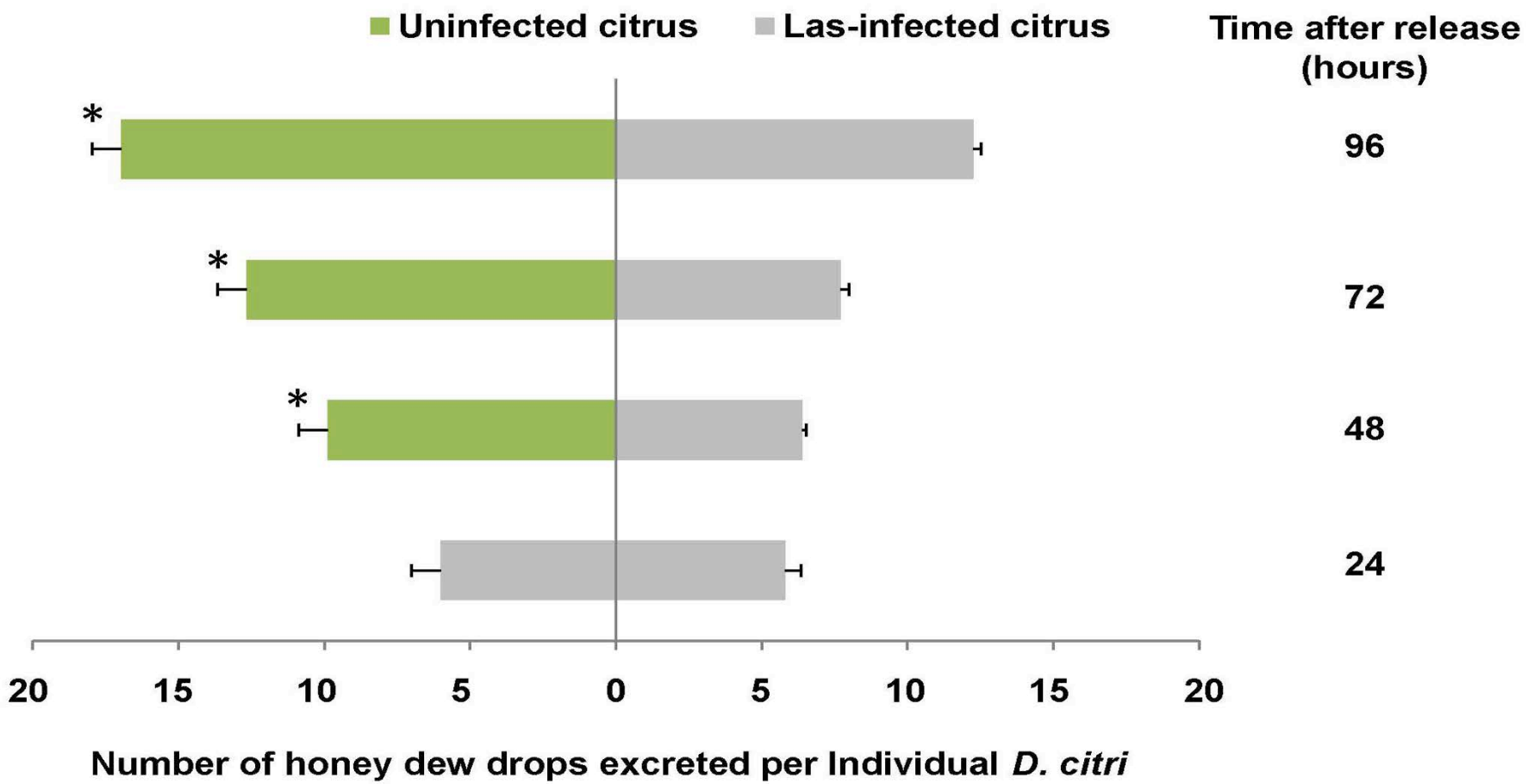
Air delivery system

Volatile collection chamber

- **Charcoal-purified and humidified air drawn over plants and pulled out at a rate of 300 mL min^{-1} through a Super-Q adsorbent trap for 24 hrs.**

- **Super-Q traps rinsed with 150 µl of dichloromethane into individual 2.0 ml clear glass vials to obtain the samples.**

Psyllids Feed Less on HLB-infected than Uninfected Plants- Potentially because they are Nutritionally Imbalanced



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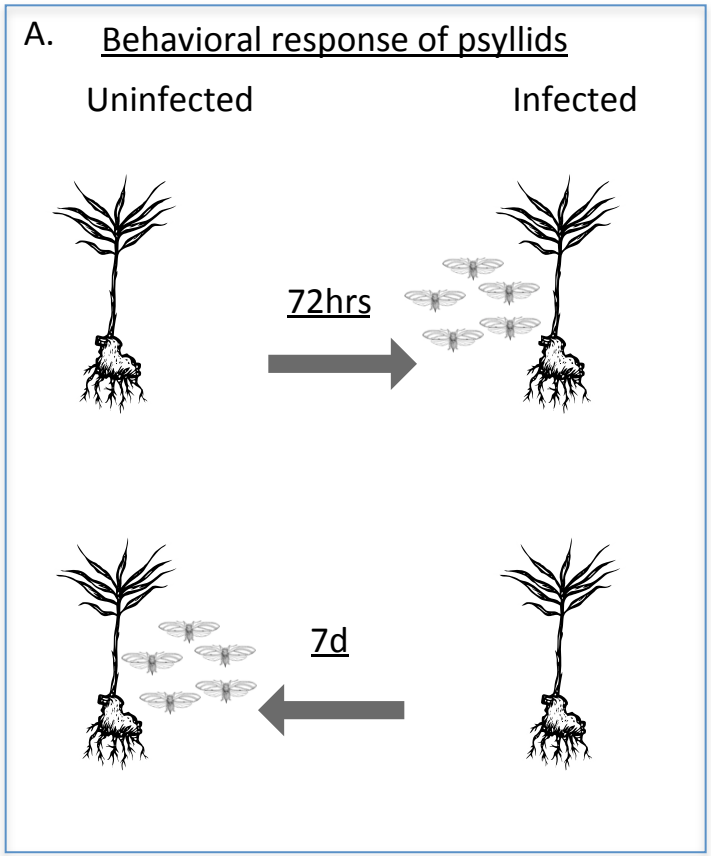

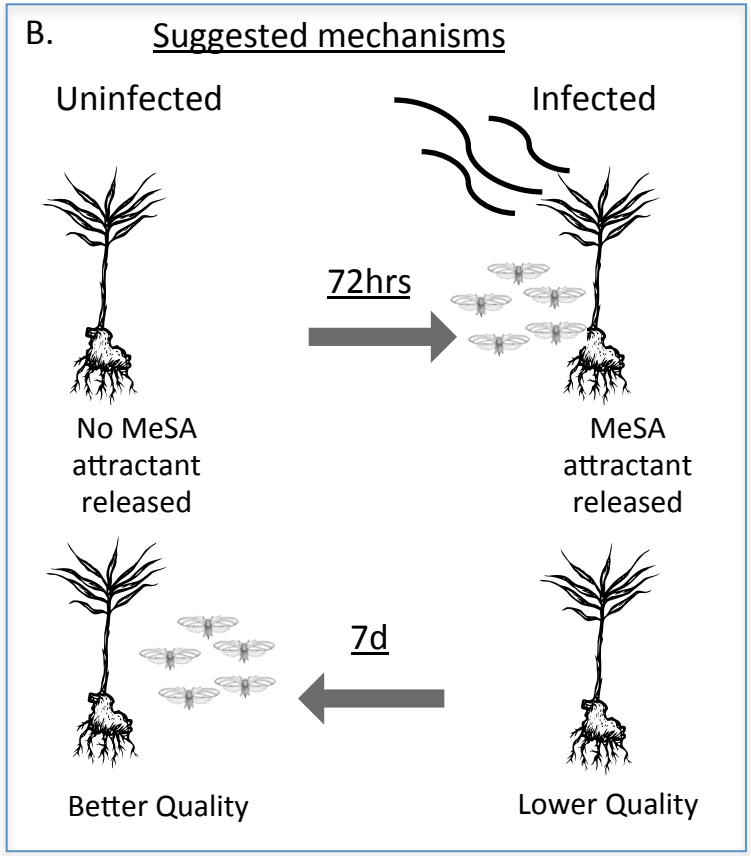

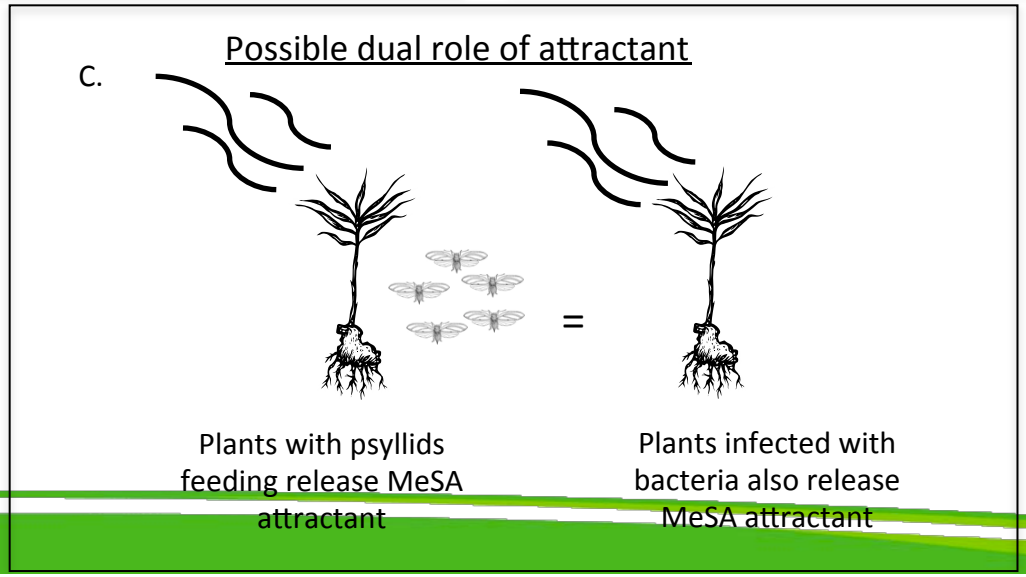

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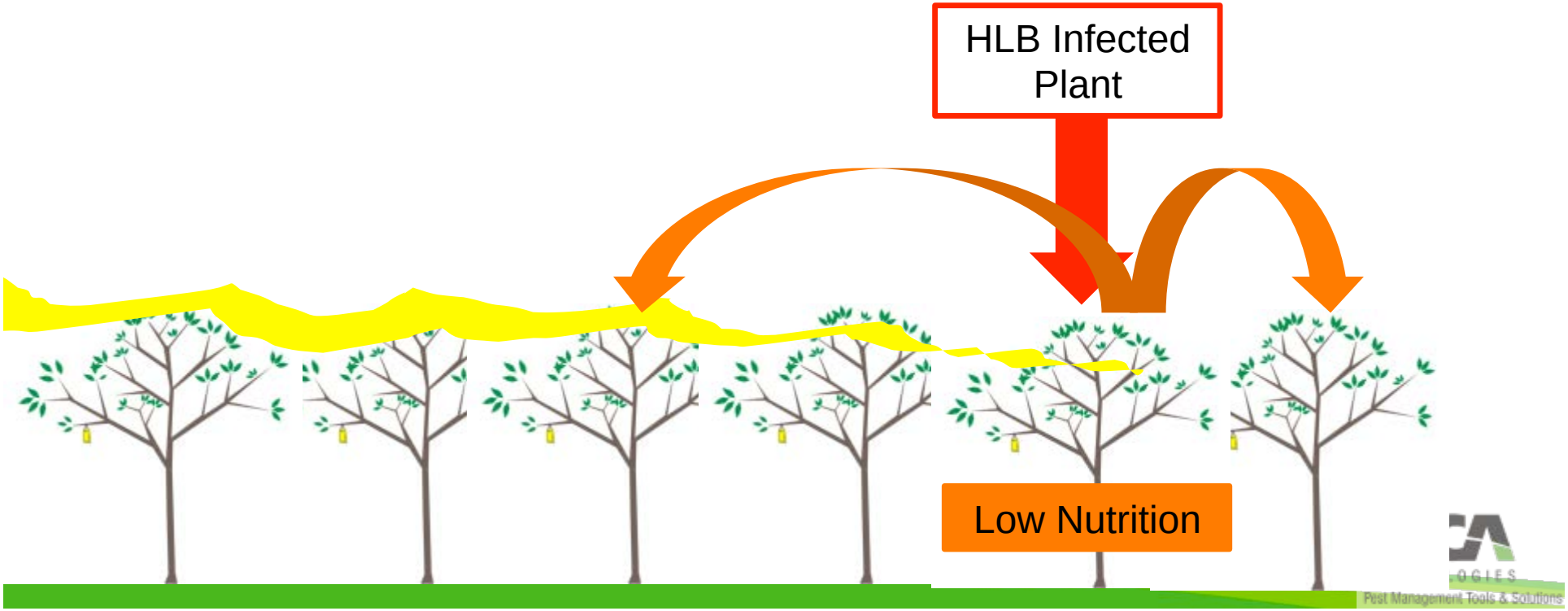
Non-infected citrus

HLB infected citrus (releasing MeSA)



It seems that:

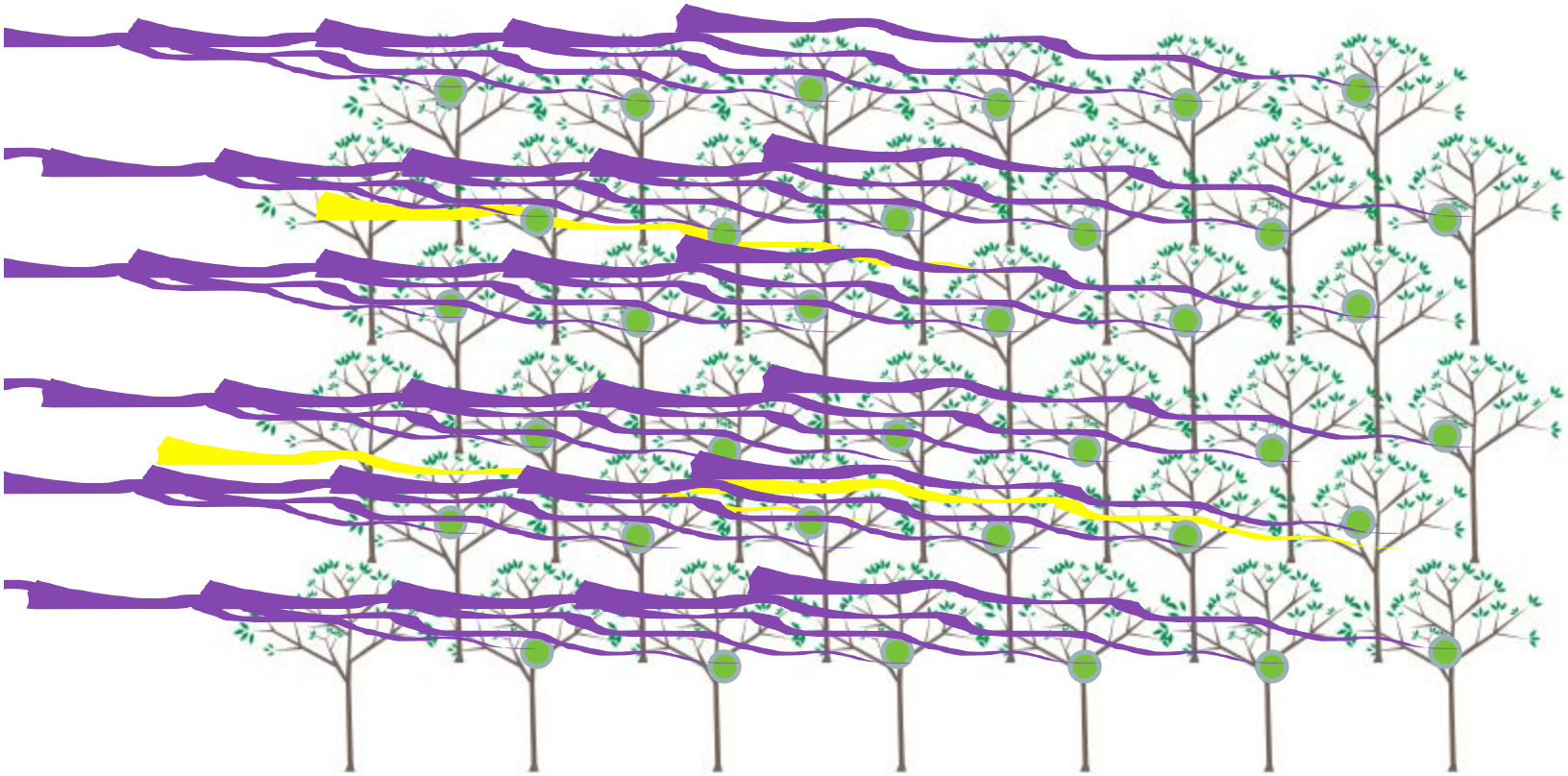
- HLB MANIPULATES the PLANT to emit MeSA, which attracts ACP to suboptimal plants
- ACP feeds on infected plants, and moves on to settle on optimal,

Non-infected plants

- HLB is transmission becomes significantly faster

CAN WE DISRUPT THIS BEHAVIORAL SEQUENCE?

DISRUPTION



Competitive Attraction= Numerous Synthetic Plumes Compete for the Available Time of the ACP Against Natural Plumes

CONCLUSIONS

- **ACP has a sex pheromone**, we just don't know if it will be strong enough, and if it will elicit the behavior from long distances or not
- **ACP can be repelled from DMDS treated areas**, we need to figure out how to use it in IPM
- **ACP is attracted to flush volatiles**, we don't seem to have a blend that is strong enough to be used anytime/anywhere
- **Beneficials are attracted to synthetic "damaged plant volatiles"** treated areas, it might cause a good suppression of ACP
- **HLB seems to manipulate the behavior of ACP**, creating an effective mechanism for infection of healthy plants, it appears that we can disrupt this sequence
- We need to **INTEGRATE** these semiochemical solutions with IPM practices.

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