



growing  
ADVANTAGE  
The Almond Conference

# Research Updates

Gabriele Ludwig, ABC (Moderator)

Frank Zalom, UC Davis

John Beck, USDA-ARS, Albany, CA

Kris Tollerup, UCCE IPM Advisor

Andrea Joyce, UC Merced

Mark Demkovich, University of Illinois

Gloria DeGrandi-Hoffman, USDA-ARS, Carl Hayden  
Bee Center

Carolyn Breece, Oregon State University

Neal Williams, UC Davis

Dennis vanEngelsdorp, University of Maryland

Troy Anderson, Virginia Tech



A close-up photograph of several green almonds on a branch, with vibrant green leaves. The background is softly blurred, showing hints of other people and greenery, suggesting an outdoor setting like a conference or field visit.

**Frank Zalom, UC Davis**



## Insect and Mite Research

Frank Zalom

Dept. of Entomology and Nematology

University of California, Davis



## Objectives for 2014-15

1. Determine treatment timing of Brigade, Intrepid, Delegate, Altacor, and Belt for NOW control in spring with comparison to male pheromone trap captures and egg trap captures.
2. Evaluate residual efficacy of these products.
3. Determine if low temperatures delay mating or oviposition by NOW females.

## Treatment Timing

### Methods:

- Ripon site in both 2013 and 2014
- NOW and PTB pheromone traps
- NOW black egg traps
- 20 mummies per strand; 8 strands per treatment
- Weekly treatment dates



## Treatment Timing

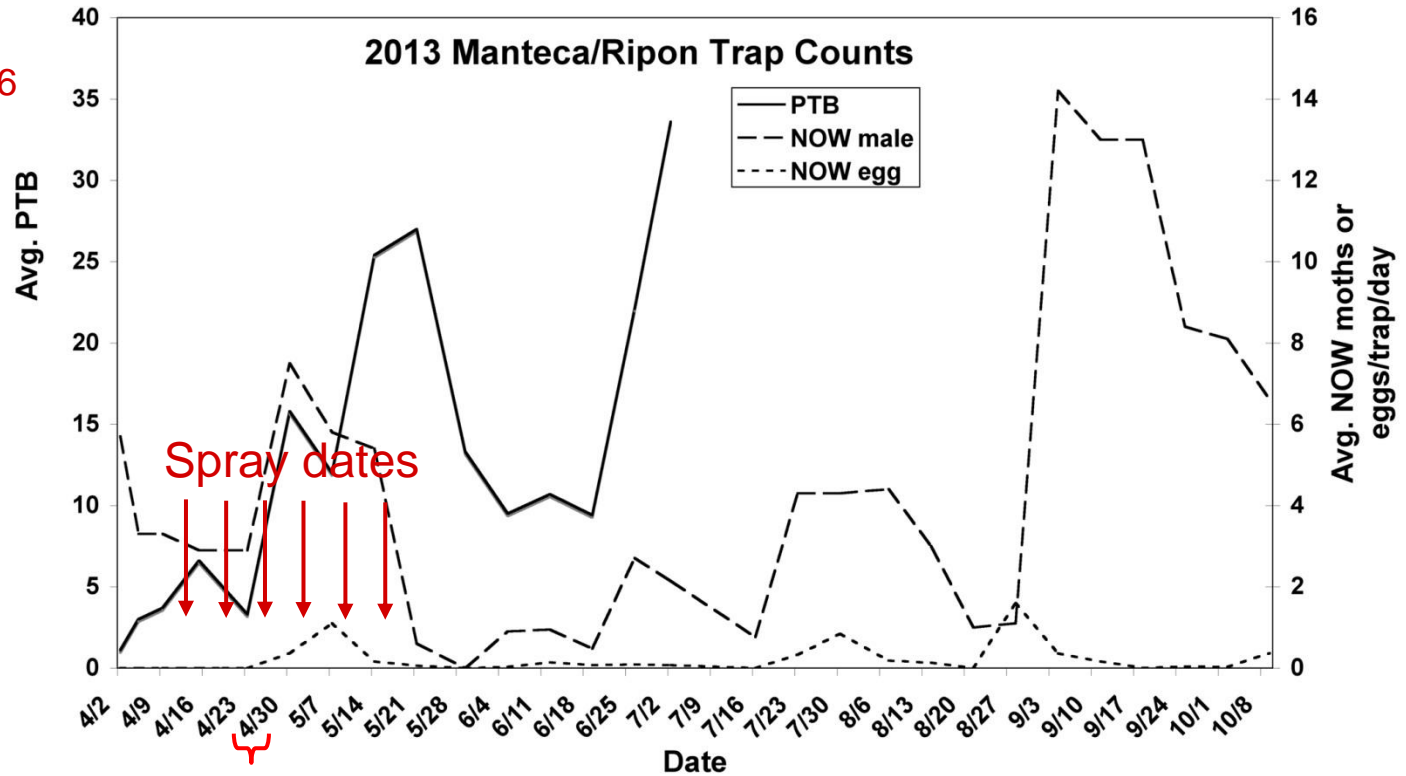
Monitoring and treatment guidelines:

- Peach twig borer – pheromone trap + Trece ‘long life’ lure  
treatment timing = 400 DD after biofix (first moth capture)
- Navel orangeworm – Pheromone trap + Suterra NOW lure
- Navel orangeworm – Black egg trap + almond presscake and oil bait (2013, used without oil in 2014)

treatment timing = 100 DD after biofix (eggs on 50% of traps for 2 consecutive weeks)

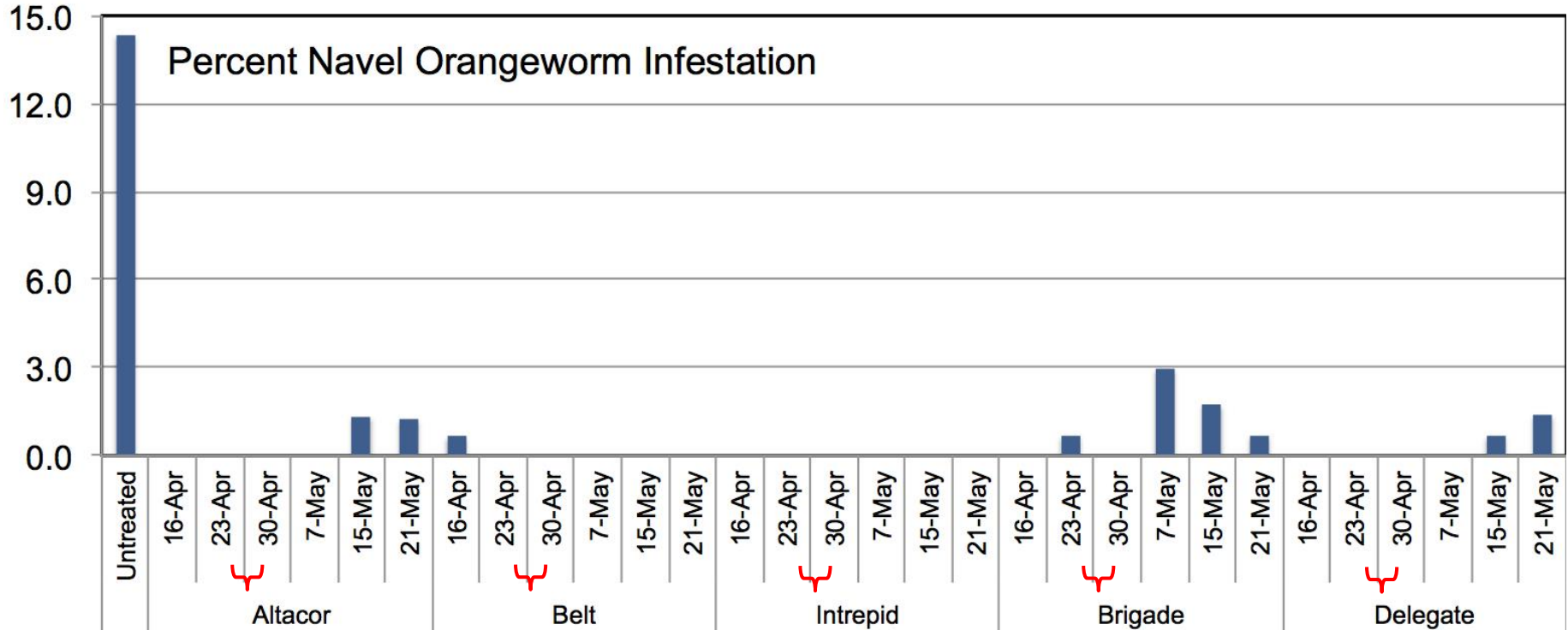
# Treatment Timing and Trapping - 2013

400 PTB DD = April 28  
100 NOW DD = April 26  
(based on egg traps)



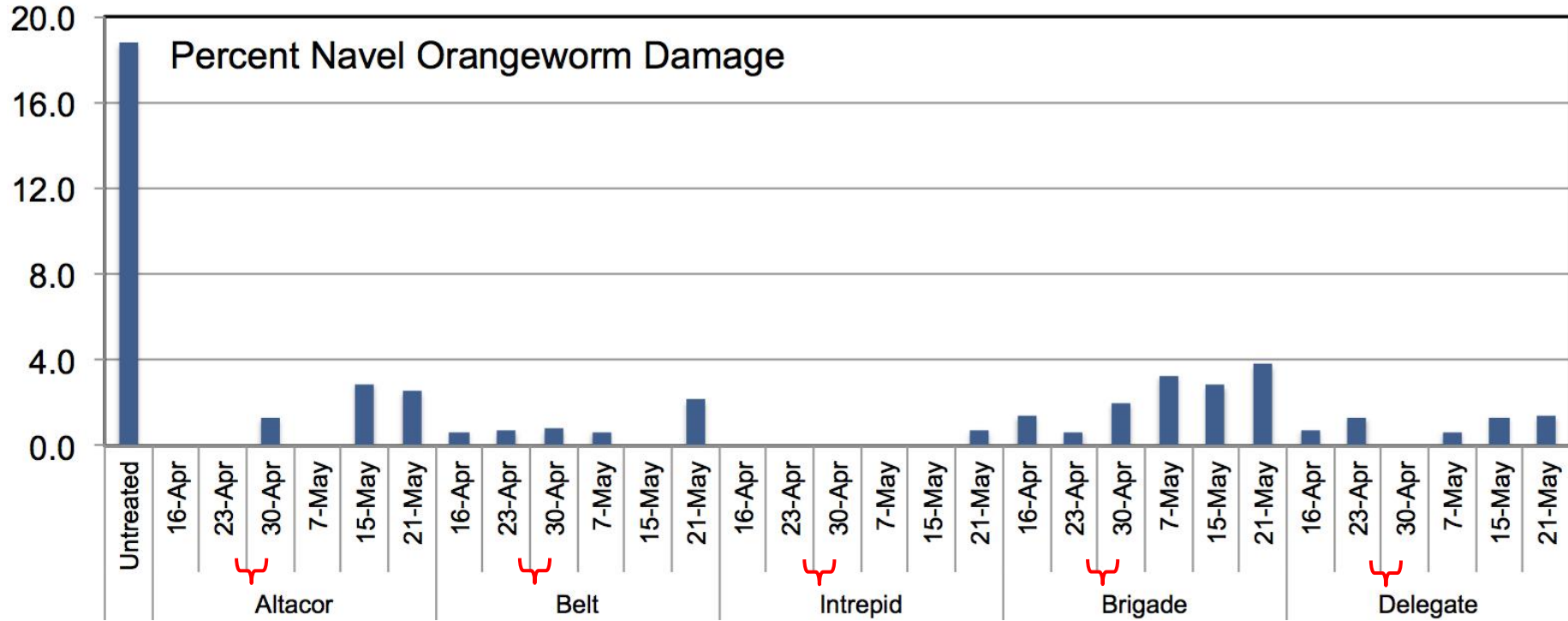


# Treatment Timing and Trapping - 2013



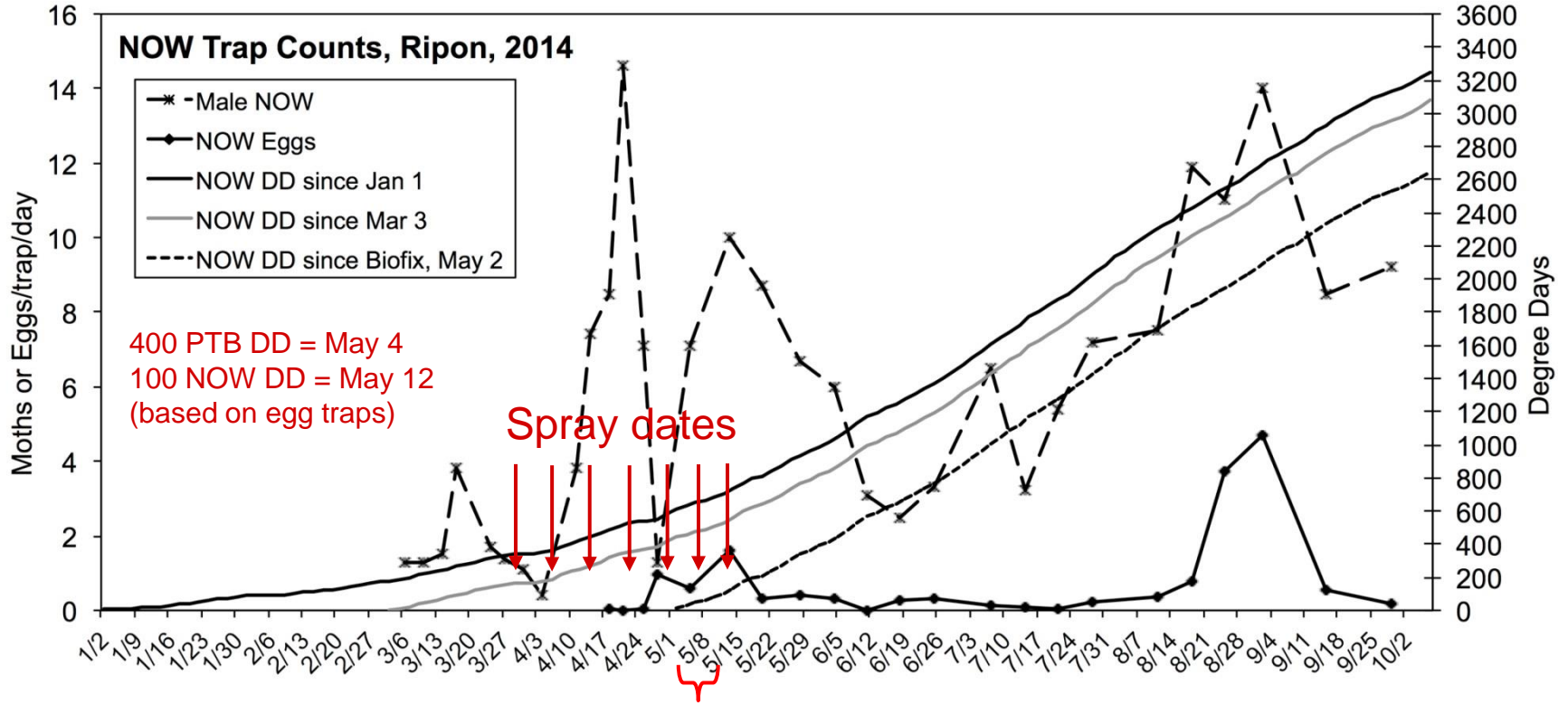
ANOVA statistics,  $F=8.1816$ ;  $df=30,258$ ;  $P<0.0001$

# Treatment Timing and Trapping - 2013

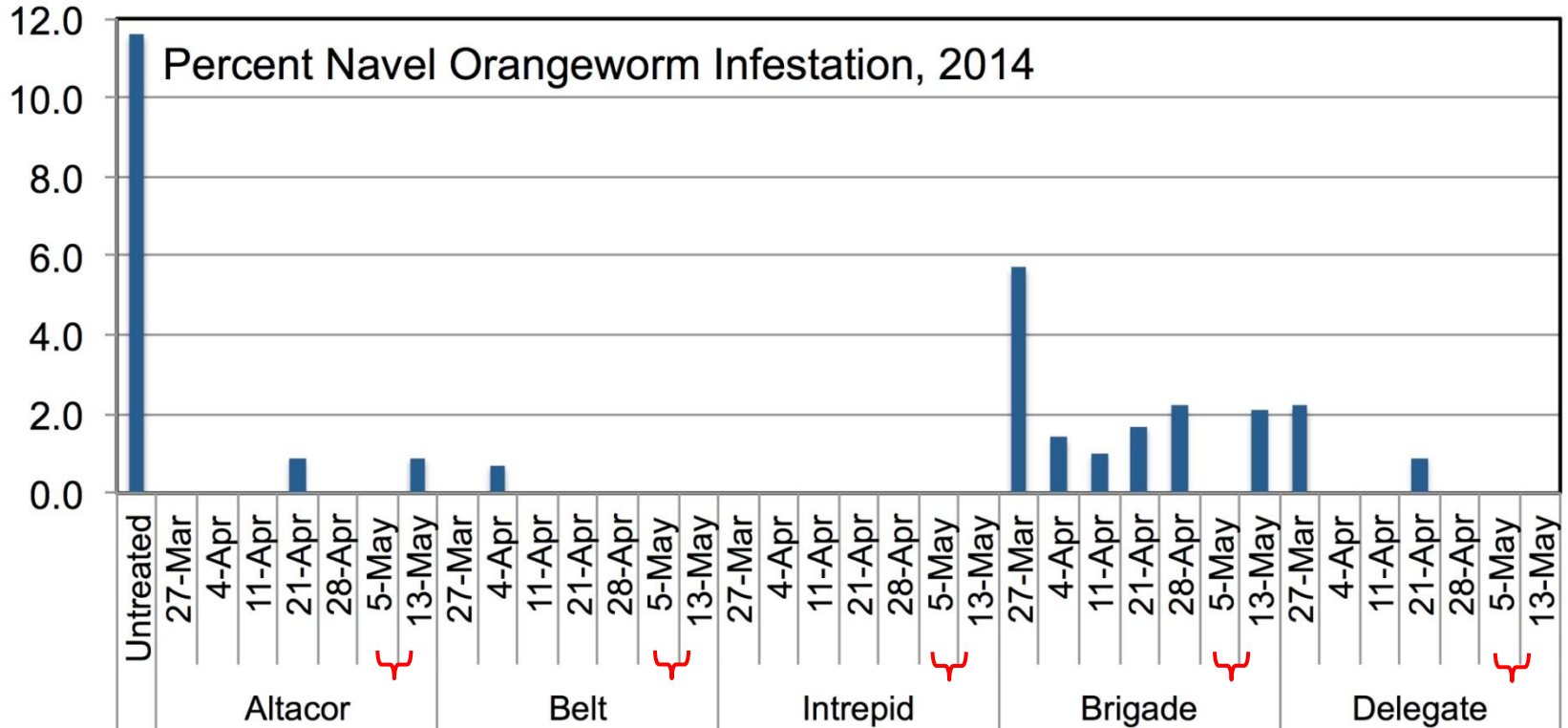


ANOVA statistics,  $F=10.9699$ ;  $df=30,258$ ;  $P<0.0001$

# Treatment Timing and Trapping - 2014

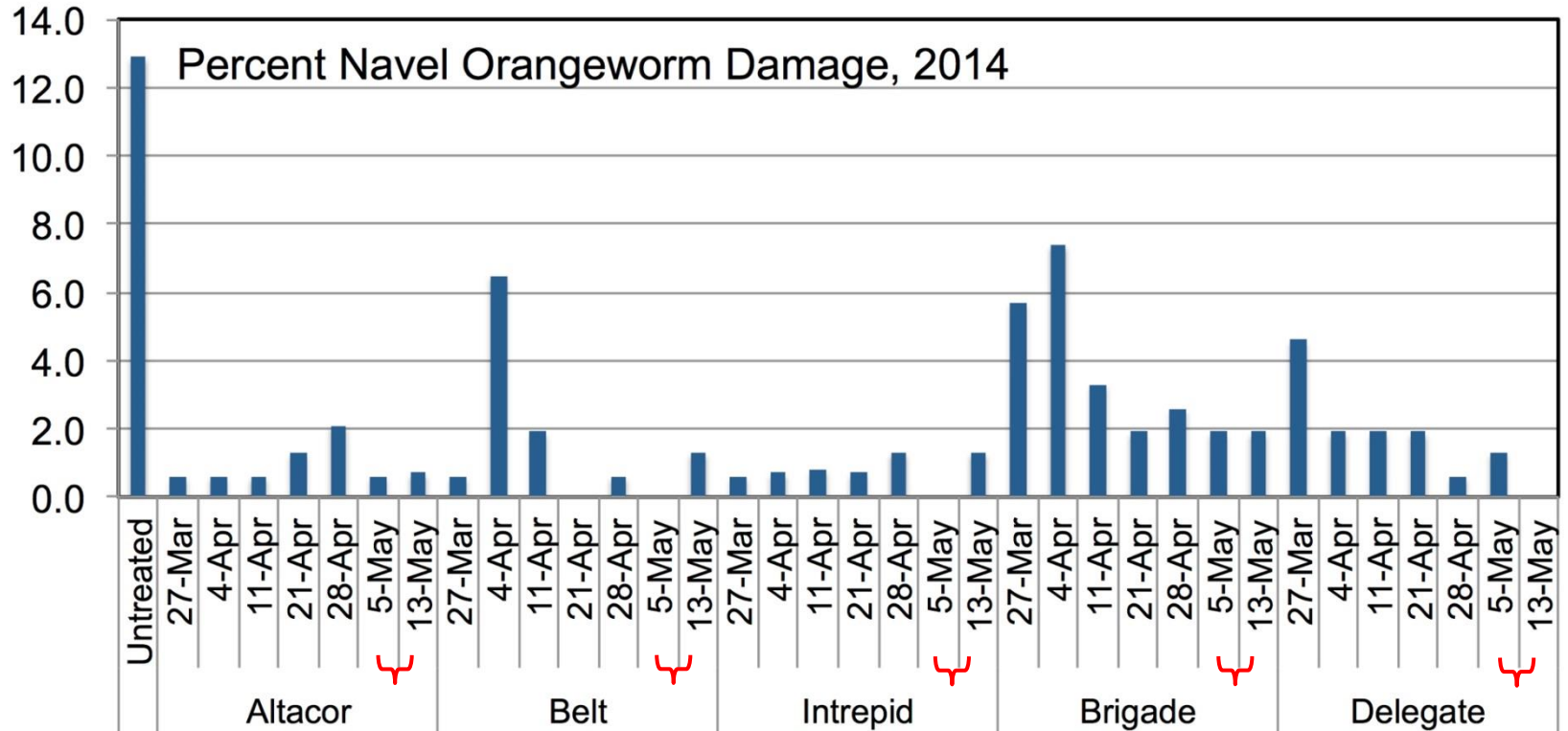


# Treatment Timing and Trapping - 2014



ANOVA statistics,  $F=5.0621$ ,  $df=35,317$ ,  $P<0.0001$

# Treatment Timing and Trapping - 2014

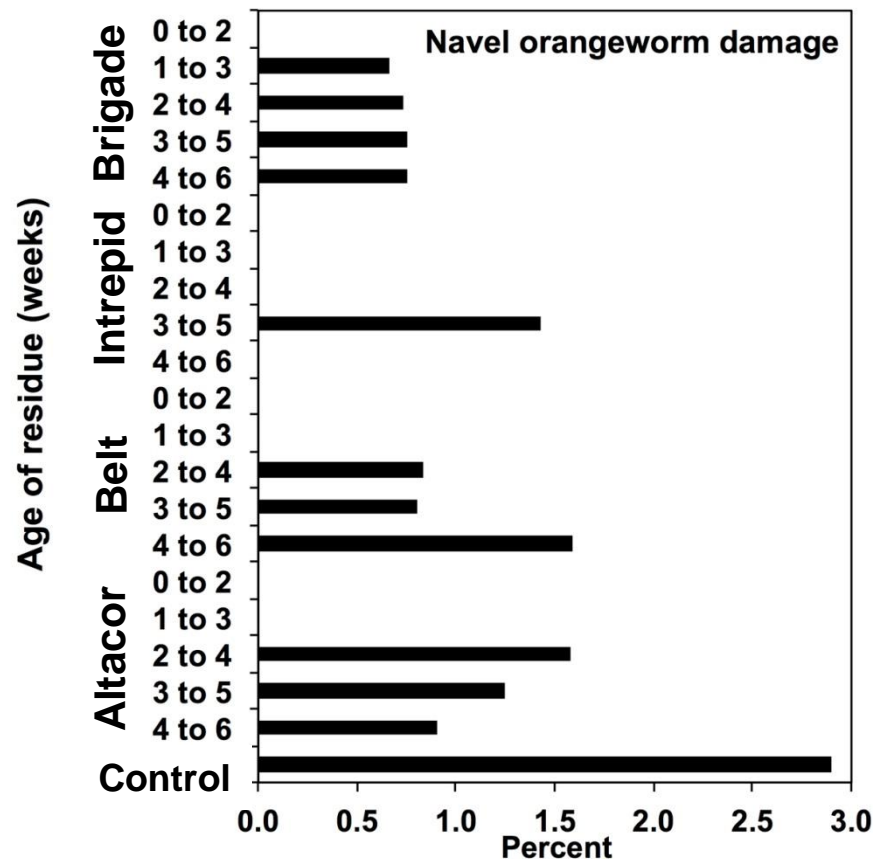


ANOVA statistics,  $F=5.3717$ ,  $df=35,317$ ,  $P<0.0001$

## Residual Activity

Average percent navel orangeworm damage resulting from nuts pre-treated weekly over a six week period and then simultaneously exposed to navel orangeworm oviposition for a two week period in a commercial almond orchard near Ripon in May.

The period when residues were sufficient to avoid infestation was about 2 weeks for Brigade, 4 weeks for Intrepid, 3 weeks for Belt, and 3 weeks for Altacor.



Visit our poster for additional information on:

- Insecticide treatment timing and efficacy for navel orangeworm
- Insecticide residual activity for navel orangeworm
- Sprayer coverage
- Navel orangeworm preferential infestation of previously-infested mummies

Thank you



**John Beck, USDA-ARS, Albany, CA**





# Host Plant Volatile Blends to Monitor NOW Populations

John J. Beck & Bradley S. Higbee



# Synthetic Host Plant Volatile Blend

JOURNAL OF  
**AGRICULTURAL AND  
FOOD CHEMISTRY**

*J. Agric. Food Chem.* **2012**, *60*, 8090-8096

Article

pubs.acs.org/JAFC

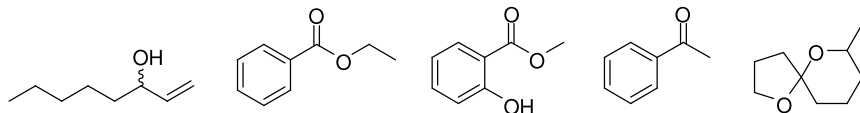


## Hull Split and Damaged Almond Volatiles Attract Male and Female Navel Orangeworm Moths

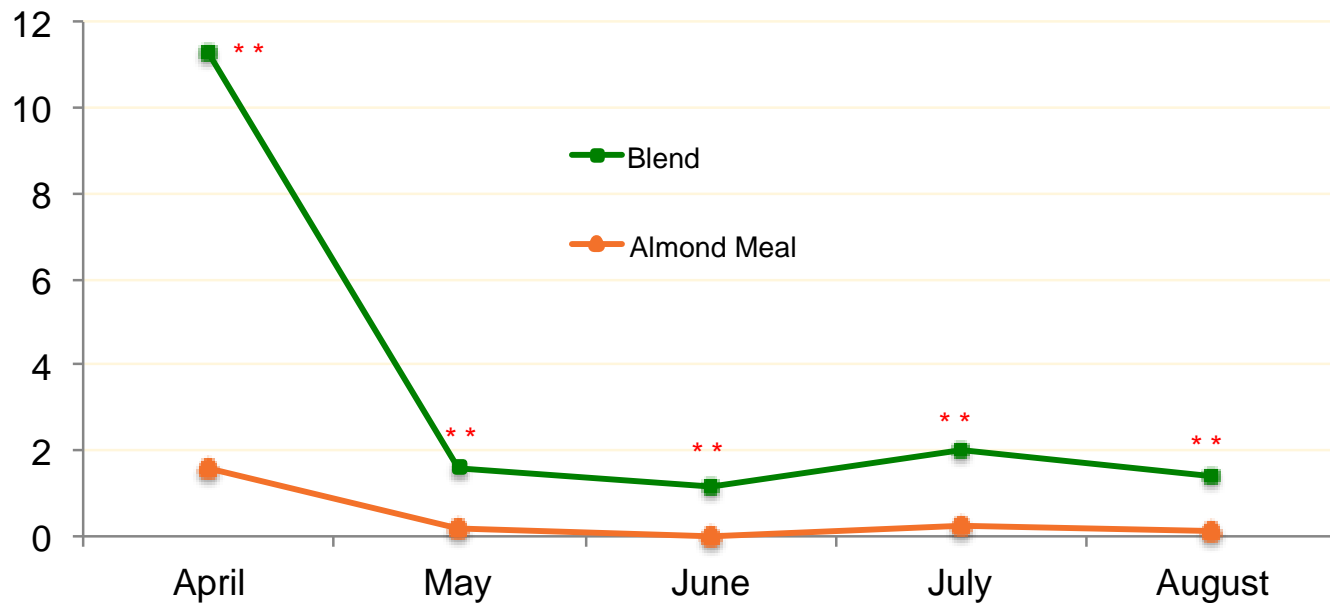
John J. Beck,<sup>\*,†</sup> Bradley S. Higbee,<sup>‡</sup> Douglas M. Light,<sup>†</sup> Wai S. Gee,<sup>†</sup> Glory B. Merrill,<sup>†</sup> and Jennifer M. Hayashi<sup>†</sup>

<sup>†</sup>Plant Mycotoxin Research, Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710, United States

<sup>‡</sup>Paramount Farming Co., 33141 E. Lerdo Highway, Bakersfield, California 93308, United States

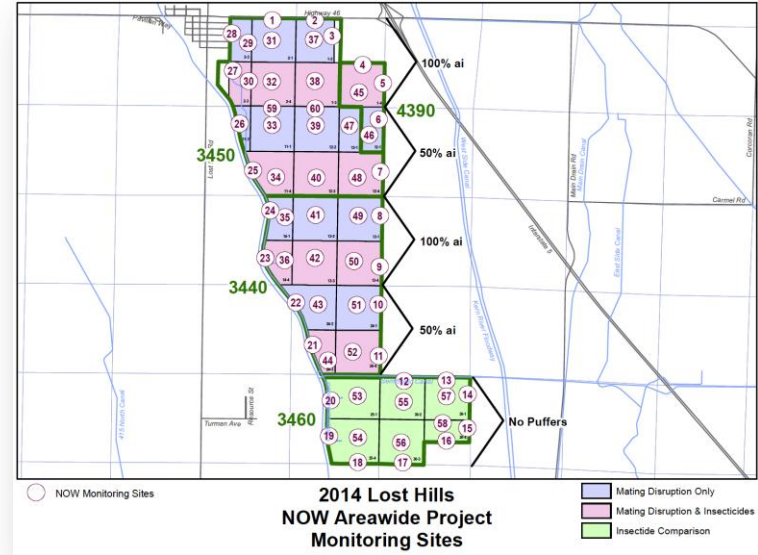
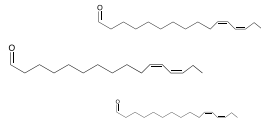


## Trap Capture Data 2011 – Conventional Orchard Male and female moths captured/trap/week

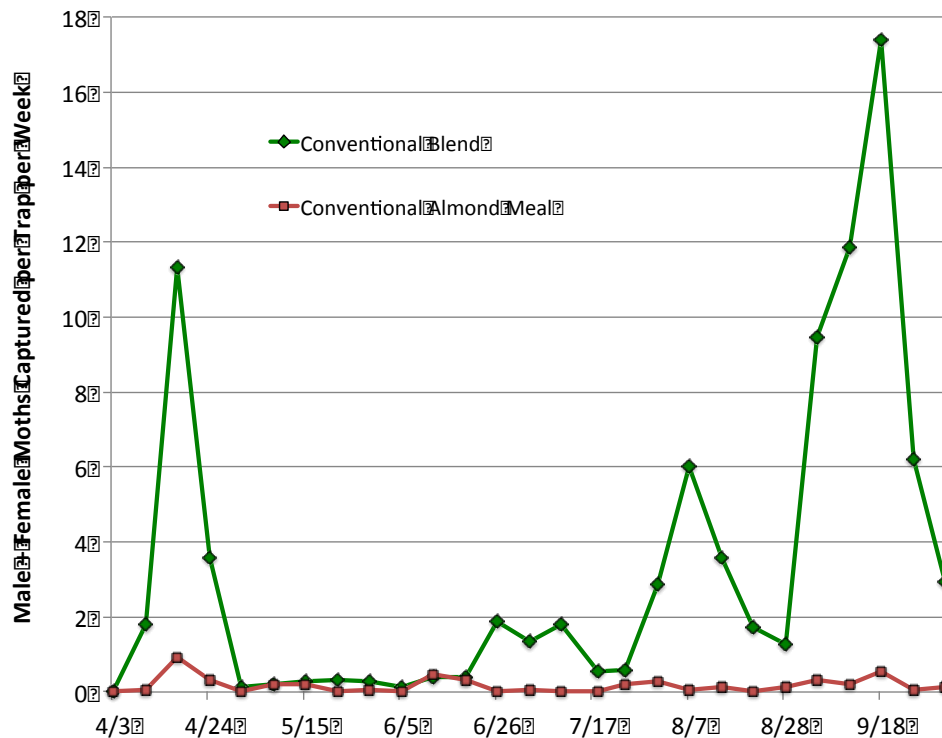


# “The Blend”

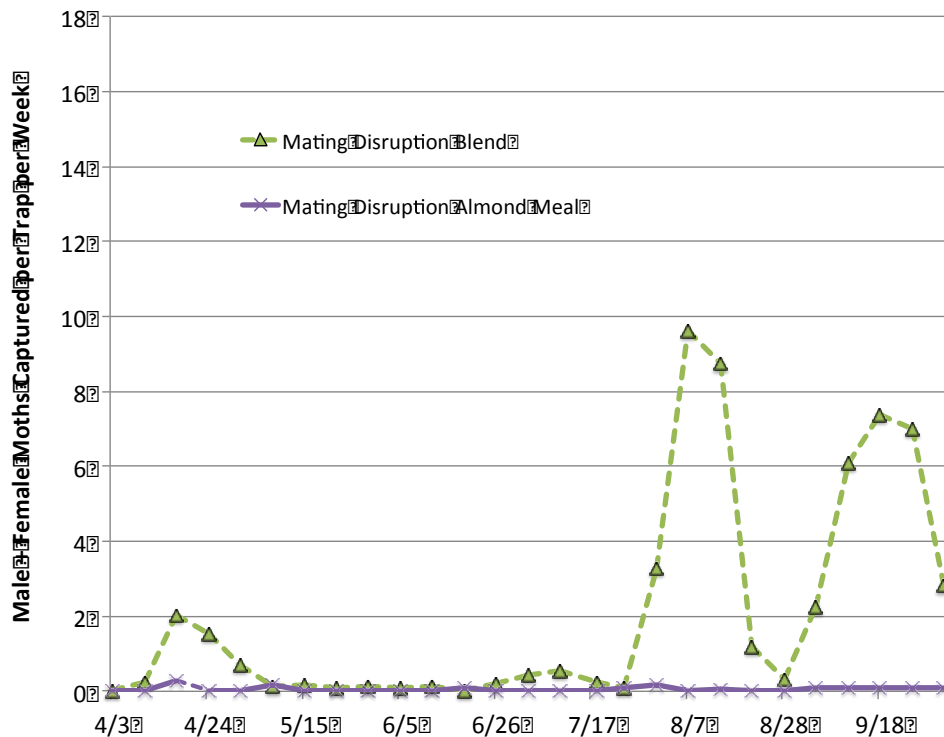
- Superiority over almond meal proven in conventional orchard
  - 2011
  - 2012
  - 2013
- Will “The Blend” maintain sensitivity and resolution in a mating disruption-treated orchard



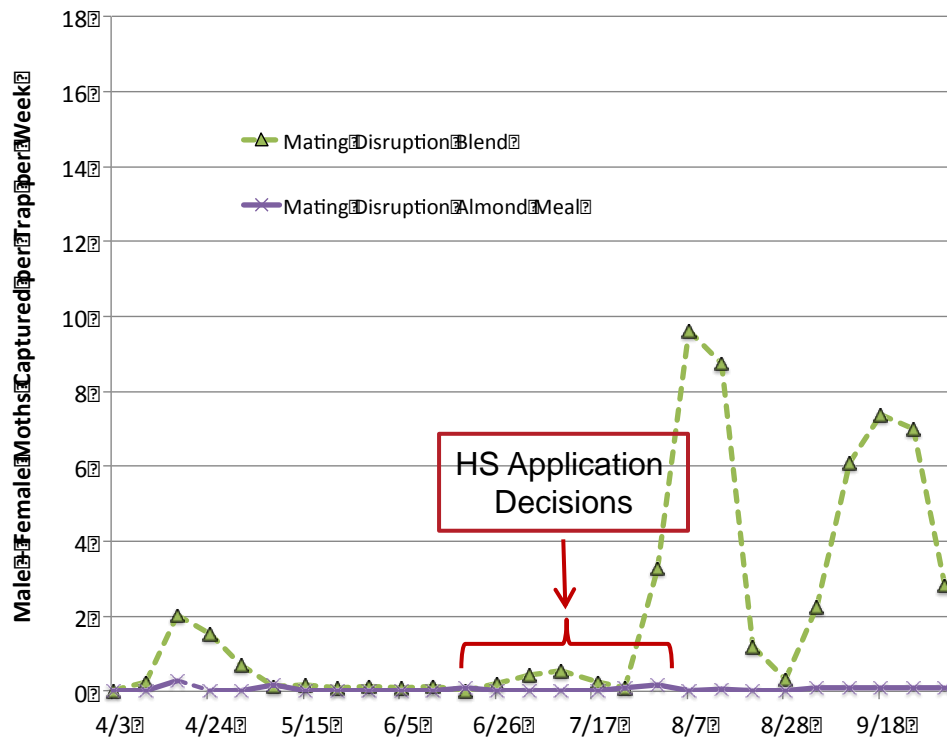
# 2014 Lost Hills Areawide Project – Conventional Orchard



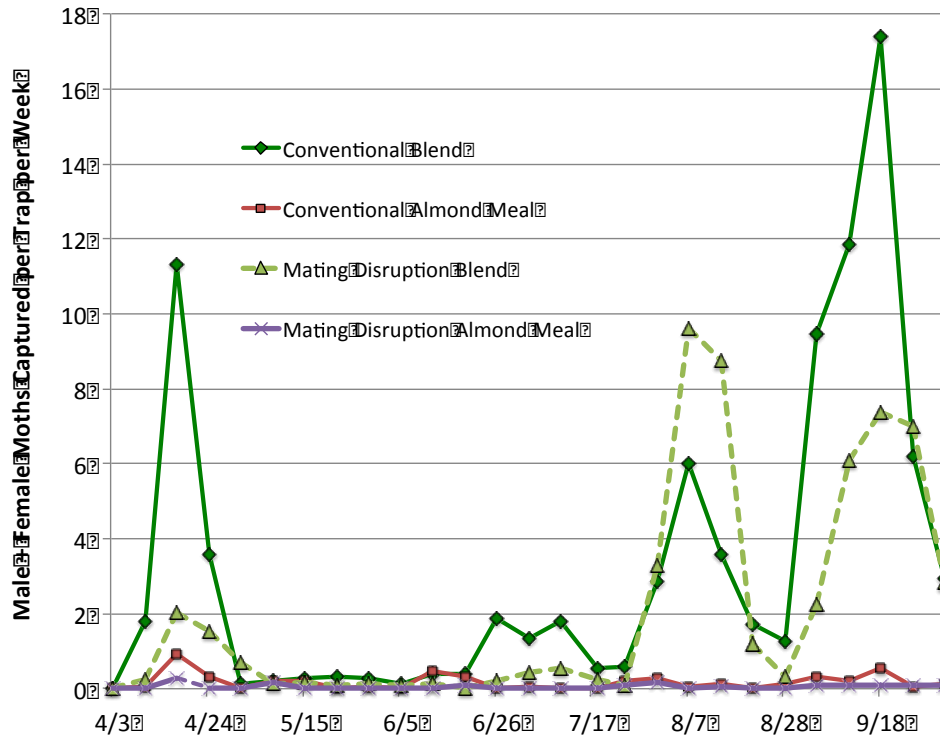
# 2014 Lost Hills Areawide Project - Mating Disruption Orchard



# 2014 Lost Hills Areawide Project - Mating Disruption Orchard



# 2014 Mating Disruption cf: Conventional





## Blend in MD and Conventional

- Provides more sensitive population dynamics information in MD environments
  - relative to sex pheromone or almond based attractants
- Interior versus exterior captures valuable for identifying risk from outside sources
- Correlations to damage in both conventional and mating disruption orchards *being analyzed from 1<sup>st</sup> year*
- Need 2-3 years of data



# Pheromone and Host Plant Volatiles for NOW Monitoring

Ring Cardé

UNIVERSITY OF CALIFORNIA  
**UCRIVERSIDE**



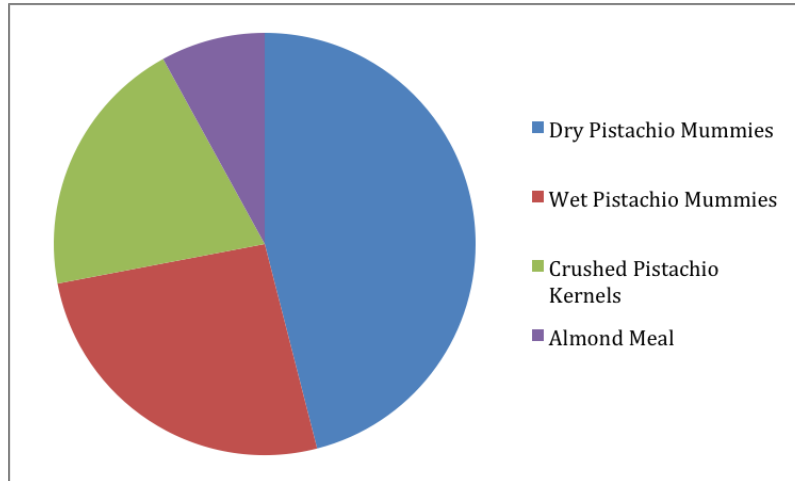
## Host Plant Volatiles to Attract Both Sexes

- Lab-based behavioral bioassay to assess attractancy
- No-exit capture system to bioassay:
  - Substrates (tissue-based matrices)
    - Almond meal (control)
    - Almond and pistachio mummies
  - Single odors
  - Synthetic blend



## Host Plant Volatiles to Attract Both Sexes

- Results from tissue-based assay
- Identification of volatiles that induce attraction is underway



## Host Plant Volatiles to Enhance Male Attraction to Pheromone?

- Host plant volatiles are known to enhance attraction to pheromone in:
  - Codling moth
  - European grape vine moth
  - Other noctuid species
- Wind-tunnel bioassay
- Determine if electrophysiological active host plant volatiles or volatile blends can synergize male NOW attraction to pheromone





Thank You!

# Kris Tollerup, UCCE IPM Advisor





# Overview of Research and Objectives

Kris Tollerup, University of California  
Cooperative Extension Advisor, IPM,  
Kearney Agricultural Research and  
Extension Center



# State-Wide Monitoring Study to Determine Relationship between Navel Orange Worm Egg and Male Moth Capture

- Evaluate NOW population dynamics over the almond-production region of California from the southern San Joaquin Valley (Kern County) to the Sacramento Valley region (Glenn / Tehama counties).
  - Determine biofix dates for egg-laying and male-moth capture at several sites throughout the almond-producing regions.
    - Evaluate the relationship between egg-capture and male-moth capture biofixes.
    - Evaluate relationship between intra-season male-moth and egg-laying data.
  - Evaluate applicability of the UC IPM navel orange worm degree-day model using a male-moth capture biofix.



UC Statewide IPM Project  
© 2009 Regents, University of California

# Developing an Early-Season Monitoring System for Leaffooted Bug on Almond

- Short-term (within 2014-2015 funding period).
  - Evaluate indicators that provide an early-season mechanism for estimating leaffooted bug (LFB) population densities i. e. traps.
  - Evaluate the effect of temperature on LFB mortality.
- Long-term goal is to develop an efficient and effective sampling method for LFB and stink bugs on almond.
  - Continue work to determine the aggregation cues of LFB.
- Evaluate effectiveness of various insecticides as potential tools to manage big bugs on almond / pistachio.
  - Determine longevity of various insecticides under field-weathered conditions.
  - Under laboratory conditions, determine if any of the evaluated insecticides have feeding deterrence or repellency activity.





**For more information, please see me  
at the poster session.**



**Andrea Joyce, UC Merced**

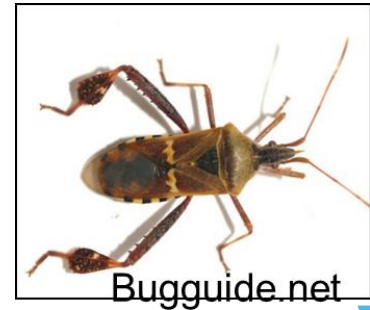
# Leaffooted Bugs and Stink Bugs in Almonds

Andrea Joyce, UC Merced



# The Problem

- Feeding causes gumming, almond drop and kernel damage
- *Leptoglossus clypealis*, *L. occidentalis* are reported from almonds, pistachios, and pomegranate
- They are occasional pests, but an early detection system is needed



# Objectives

- 1. Determine the species composition of leaffooted bugs and stink bugs on almonds and alternate host plants***
- 2. Conduct a field-cage study to assess feeding damage by leaffooted bugs on almonds***

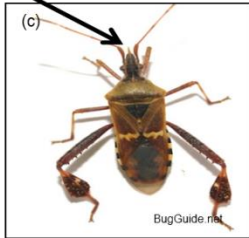
# Leaffooted Bug Collections



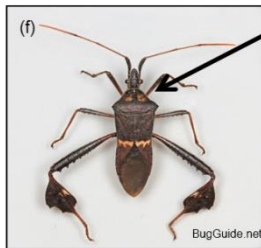
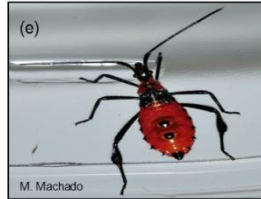


# Distinguishing these two species

*L. clypealis*



*L. zonatus*



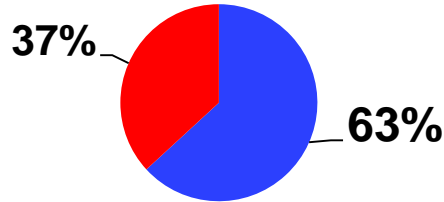
First instars of *L. clypealis* are green, *L. zonatus* are orange

Mid-sized leaffooted bugs of *L. clypealis* are copper colored, while *L. zonatus* are bright red

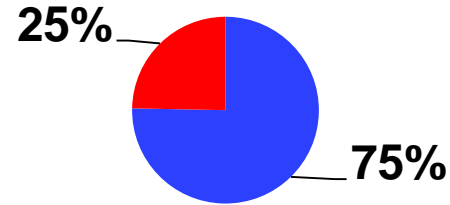
Adults of the two species are distinct

# Species Abundance by Crop

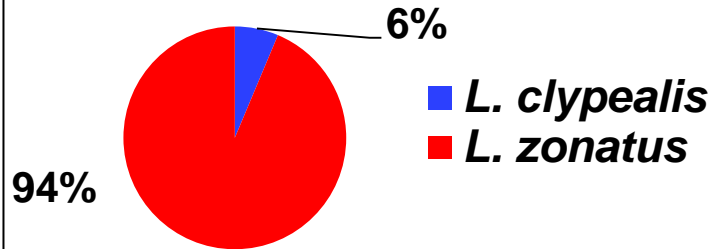
## Almond



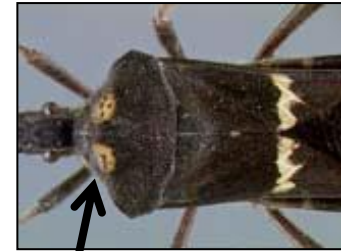
## Pistachio



## Pomegranate



*L. clypealis*  
Pointed clypeus



*L. zonatus*  
Two spots



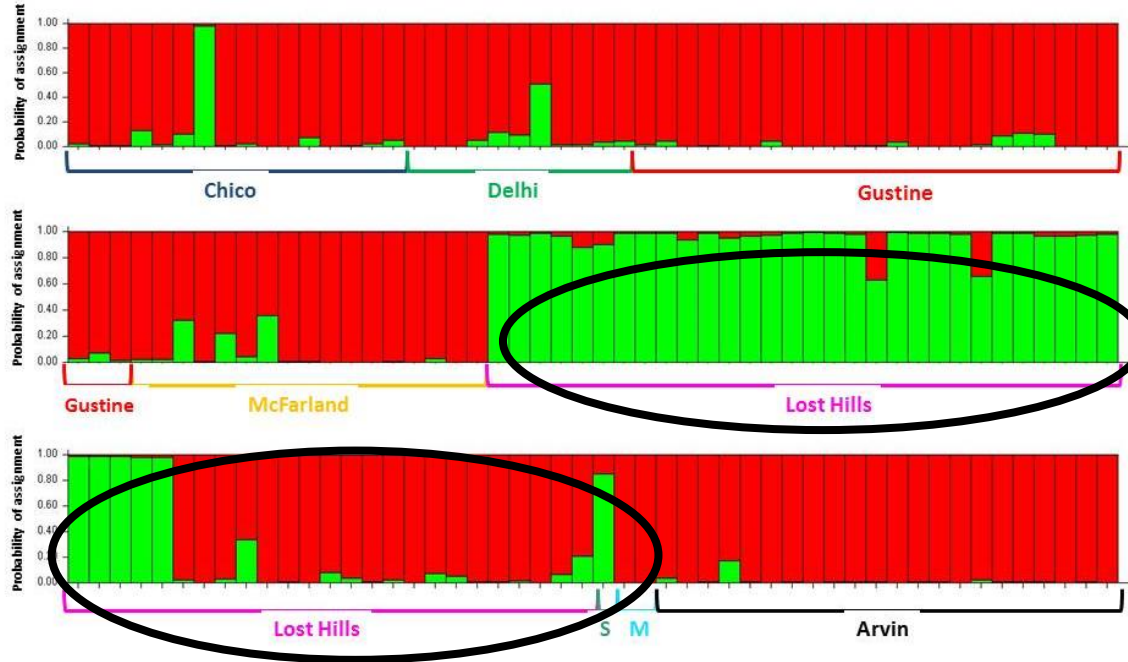
# *L. zonatus*



Most *L. zonatus* are one genotype

One site had two genotypes

*L. zonatus* were more abundant in Fall



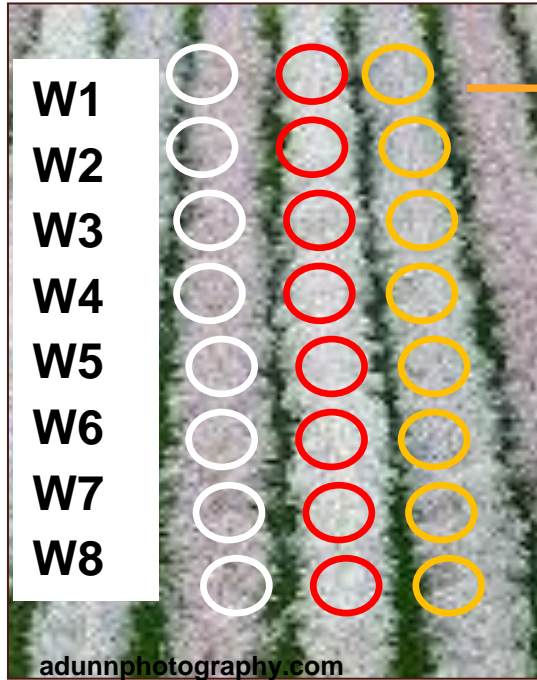
S = Shafter  
M = McKittrick

# Objectives

1. *Determine the species composition of leaffooted bugs and stink bugs in almonds and alternate host plants*
2. **Conduct a field-cage study to assess nut drop and feeding damage by leaffooted bugs on almonds**

# Field-cage Study

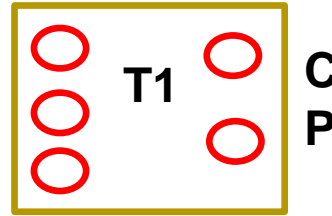
Merced



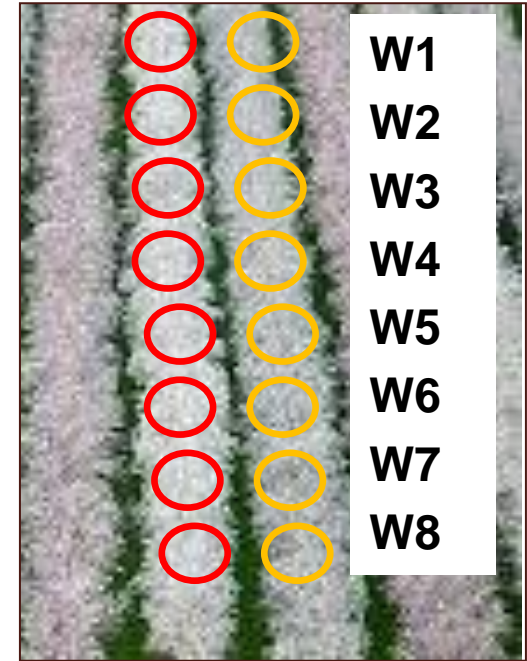
Sonora, Monterey, Carmel



Control  
Puncture  
*Lc or Lz*

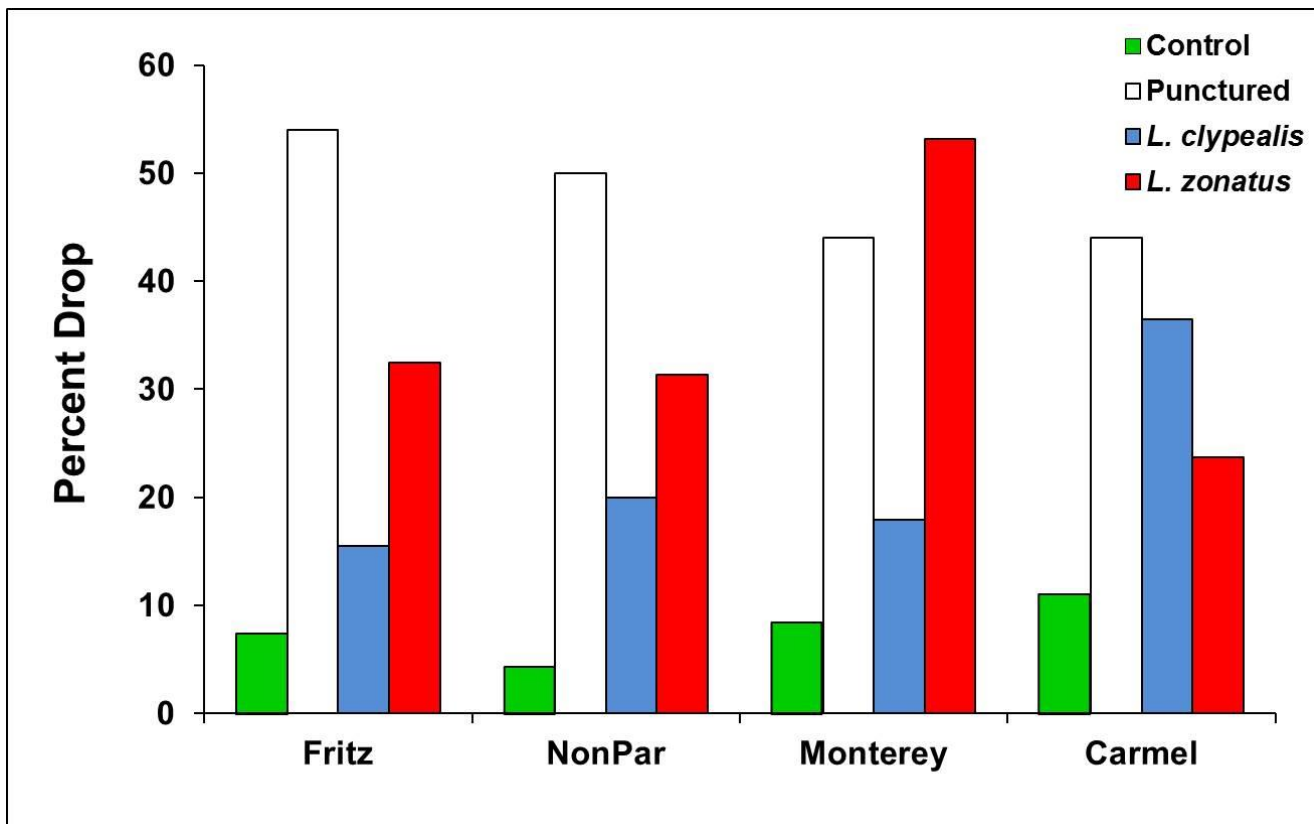


Winton

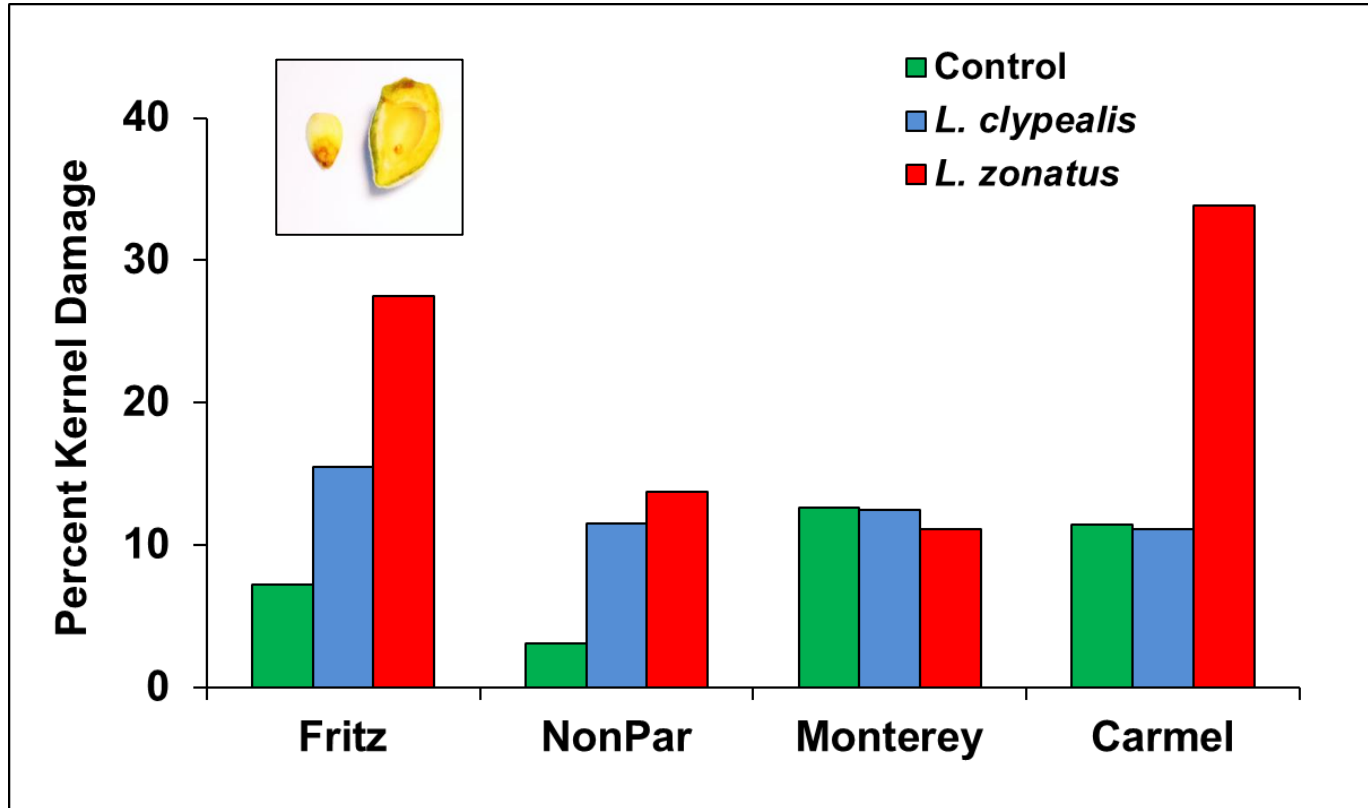


Nonpareil, Fritz

# Results-Total Almond Drop



# Almond Kernel Damage





# Conclusions

- ***L. clypealis* moves between almond and pistachio**
- ***L. clypealis* was more abundant in spring, while *L. zonatus* was more common in fall**
- **In field-cage study, both *L. clypealis* and *L. zonatus* were associated with significant almond drop and kernel damage**

# Acknowledgements

**Almond Board of California**

**UCCE: Roger Duncan, David Doll,**

**David Haviland, Joe Connell**


**Brad Higbee, Paramount Farms**

**Many PCAs and Consultants (see poster)**

**Arnold Farms, Clendenin Farms, Merced**

**UC Merced-Ashley Valle, Maria Martinez**

**Mark Demkovich**  
**University of Illinois**



# Investigating Navel Orangeworm (*Amyelois transitella*) Resistance to Pyrethroid Insecticides through Neonate and Adult Bioassays

Mark Demkovich<sup>1</sup>

Joel Siegel<sup>2</sup>

May Berenbaum<sup>1</sup>



<sup>1</sup> University of Illinois at Urbana-Champaign

<sup>2</sup> USDA-ARS, Parlier, CA

## Background and Previous Research

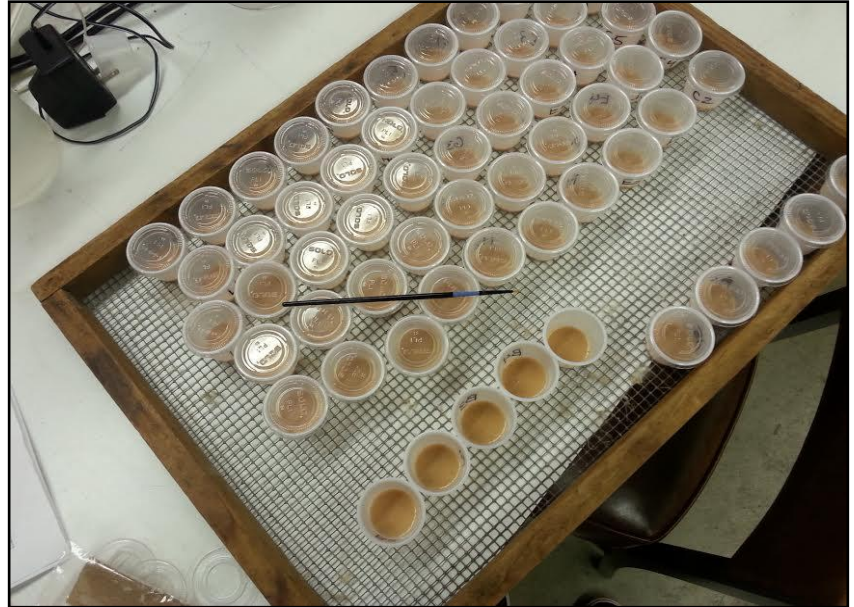
- Navel orangeworm resistance to bifenthrin was first reported at Paramount Farming Company (B. Higbee)
- June 2013- eggs were sent to the University of Illinois to establish a bifenthrin-resistant colony (R347) in the Berenbaum laboratory
- Resistance was quantified by median-lethal concentration values ( $LC_{50}$ ) to bifenthrin through neonate feeding assays, revealing a 10-fold difference between the R347 colony and susceptible laboratory (CPQ) colony
- The mechanism responsible for the 10-fold difference is likely elevated cytochrome P450 monooxygenase and esterase detoxification activity

## Research Questions

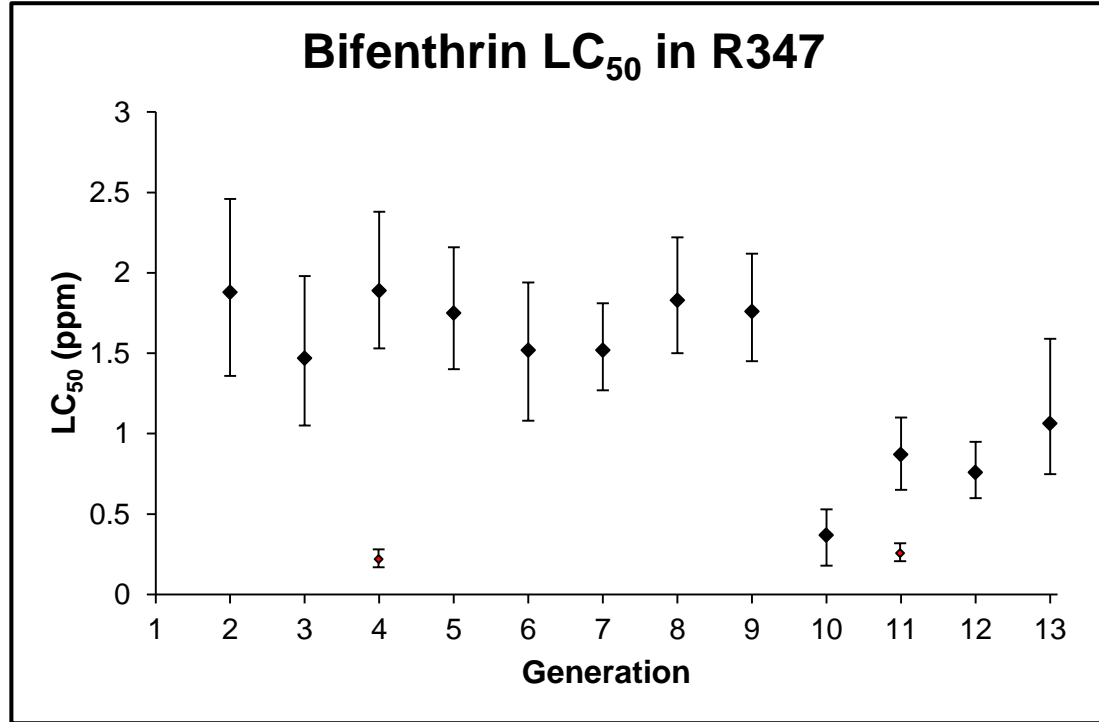
- Is resistance stable in the absence of bifenthrin selection pressure?
- Are there significant differences in the R347 and CPQ colonies when neonates and adults are sprayed with bifenthrin?
- Are there any fitness costs associated with bifenthrin resistance?

## Methods

- Bioassays (oral) on first instars across multiple generations



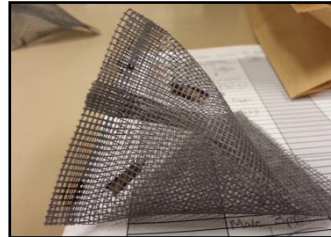
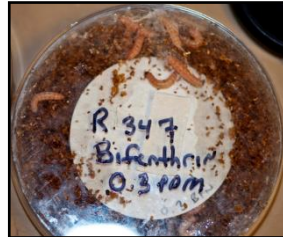
## Results



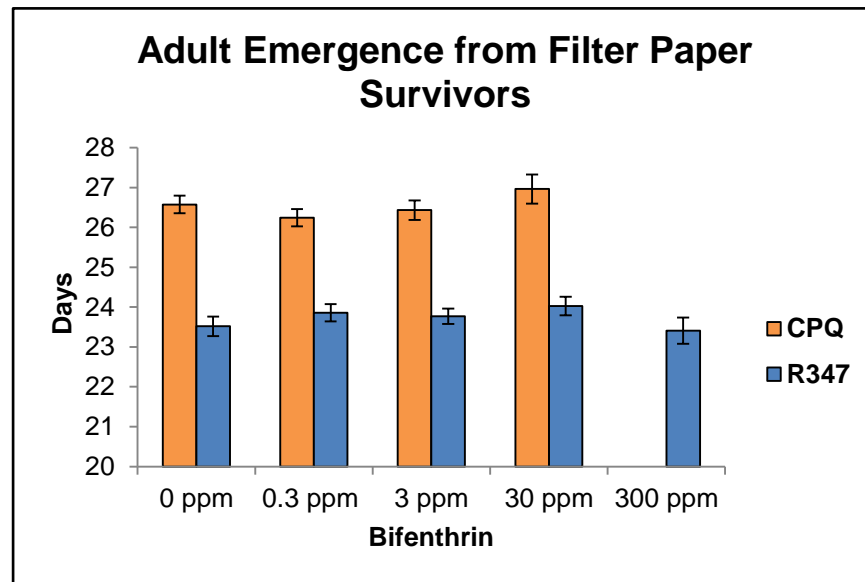
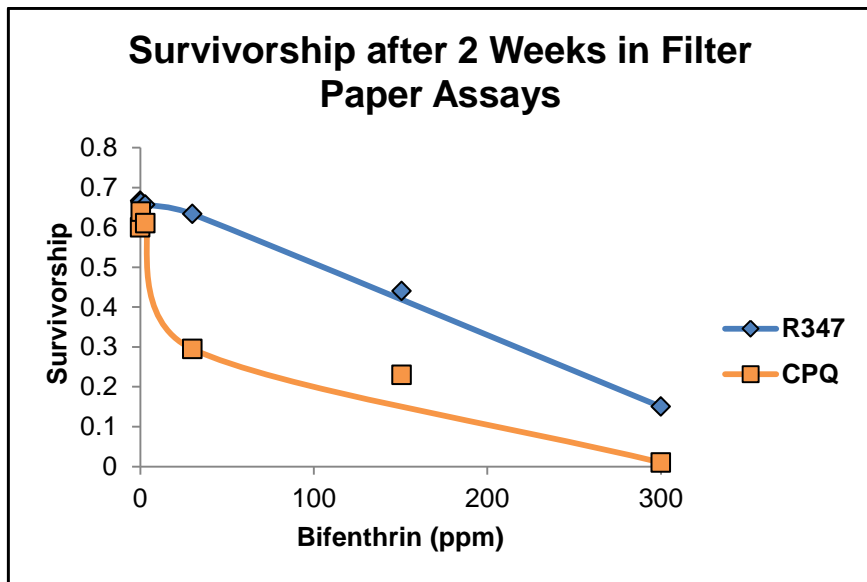


## Contact Toxicity Methods: Neonate and Adult Bioassays

- Eggs were placed on filter papers sprayed with bifenthrin at 0.3 ppm, 3 ppm, 30 ppm, and 300 ppm (organic insecticide carrier used as the control)
- Sprayed filter papers were placed in Petri dishes surrounded by wheat bran diet
- Adults were separated by sex, placed into mesh bags, and sprayed at 3 ppm with water as the insecticide carrier



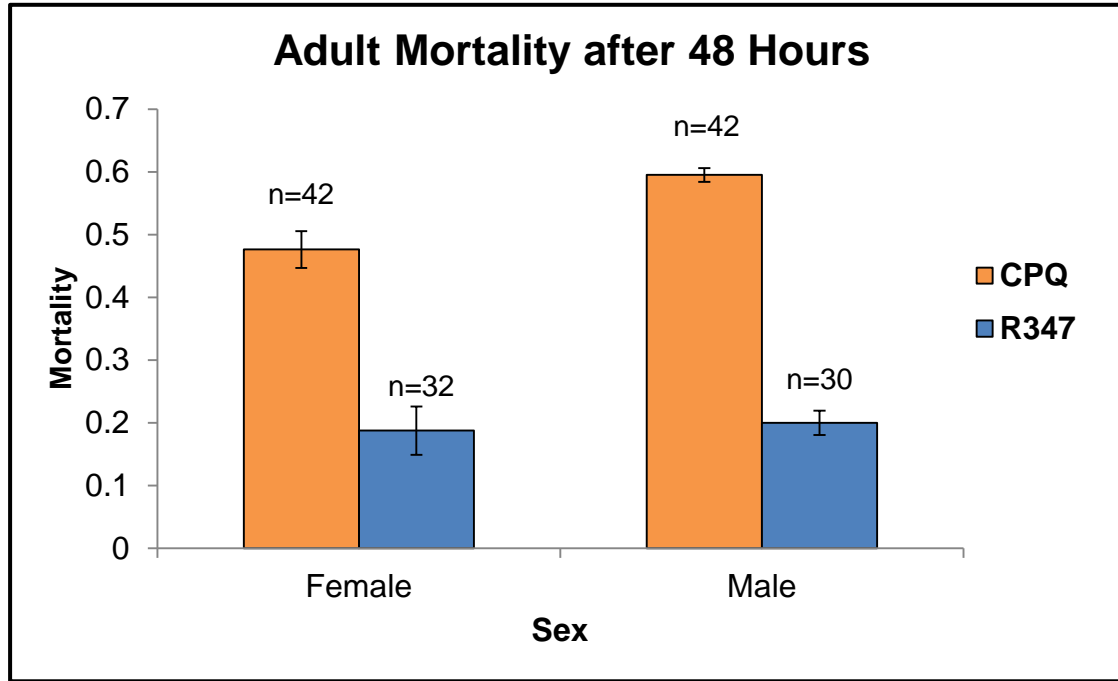
## Results: Neonate Contact Toxicity Assays



- 100 neonates per concentration
- 3 replicates in R347, 2 replicates in CPQ
- Significant differences at 30 ppm ( $P < 0.001$ ) and 300 ppm ( $P < 0.001$ ) confirmed through dummy-variable regression

- R347 completed development approximately 3 days earlier across all concentrations
- Significant differences ( $P < 0.001$ ) confirmed by t-test

## Results: Adult Spray Assays with 3 ppm Bifenthrin



Significant differences ( $P < 0.001$ ) confirmed through dummy-variable regression

## Conclusions and Future Directions

- Although filter paper assays and adult spray assays were conducted with larvae from recent generations in the R347 colony that exhibited lower resistance levels, their survivorship is still significantly greater than that of a susceptible strain at both the neonate and adult levels after bifenthrin exposure
- If navel orangeworm populations resistant to pyrethroids can complete development faster than susceptible populations, then an additional generation could potentially emerge during the growing season
- A decline in resistance over time in the absence of bifenthrin selection pressure suggests that a reduction in the use of pyrethroids could restore efficacy of the chemical class
- Future work will investigate the importance of using the newer chemistries (Altacor, Intrepid) in insecticide rotations

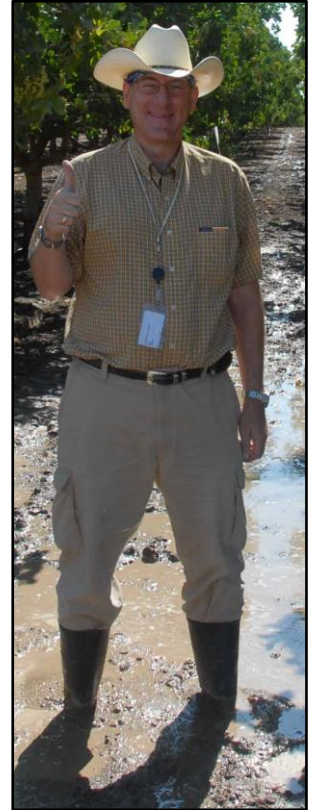
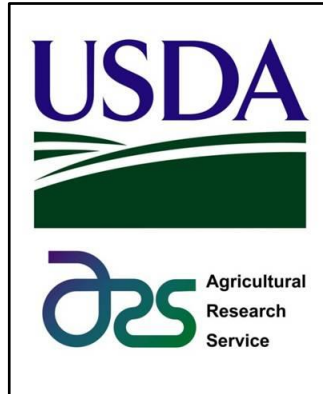
Thank You!


Phil Benedetti

Joel Siegel Laboratory Members

Berenbaum Laboratory Members

Almond Board of California



A close-up photograph of several green almonds on a branch, with vibrant green leaves. The background is softly blurred, showing more of the tree and some indistinct figures of people, suggesting an outdoor setting like a conference or field visit.

**Gloria DeGrandi-Hoffman,  
USDA-ARS,  
Carl Hayden Bee Center**

# Comparing the Effects of Protein Supplement vs. Natural Forage in Colonies Used In Almond Pollination

**Gloria DeGrandi-Hoffman**

**Carl Hayden Bee Research Center, USDA-ARS, Tucson, AZ**



# Purpose of Study

- Compare nutrient concentrations in protein supplement diets and rapini (*Brassica rapa*) pollen and determine effects on colonies





# Experimental Design

- 4 sets of 10 colonies started in November
- 9000-10,000 bees and 2 frames of brood

## Protein Supplements

Diet-1



Diet-2



## Rapini

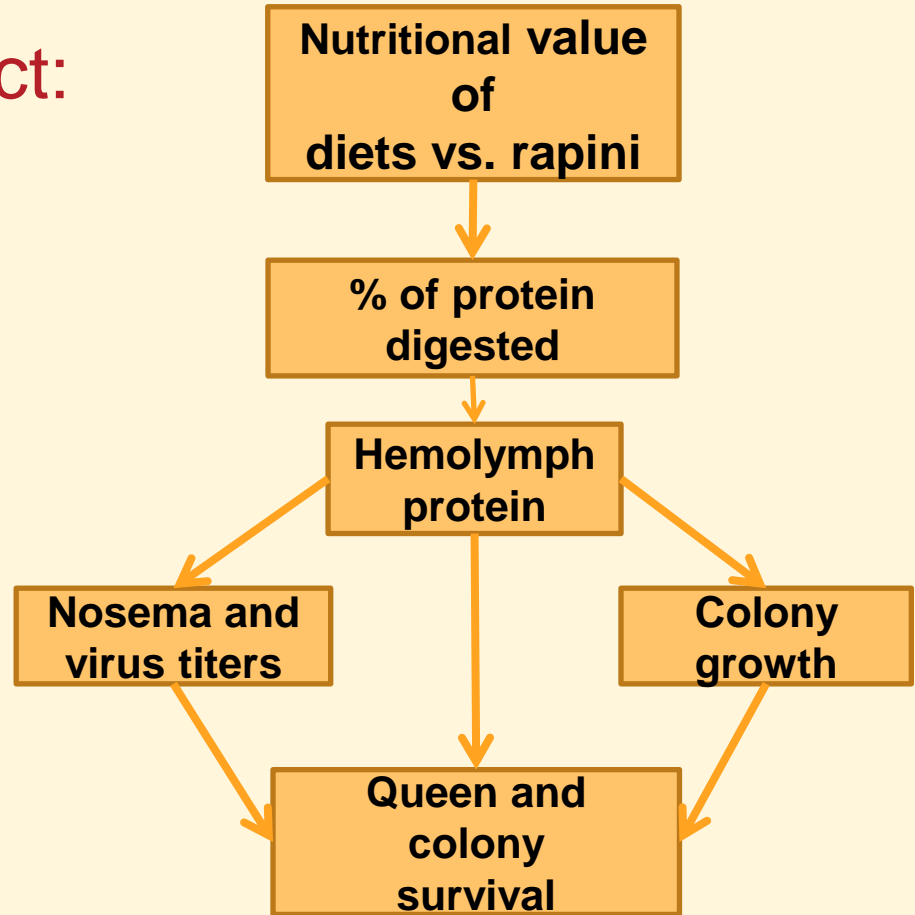
Apiary site-1

and

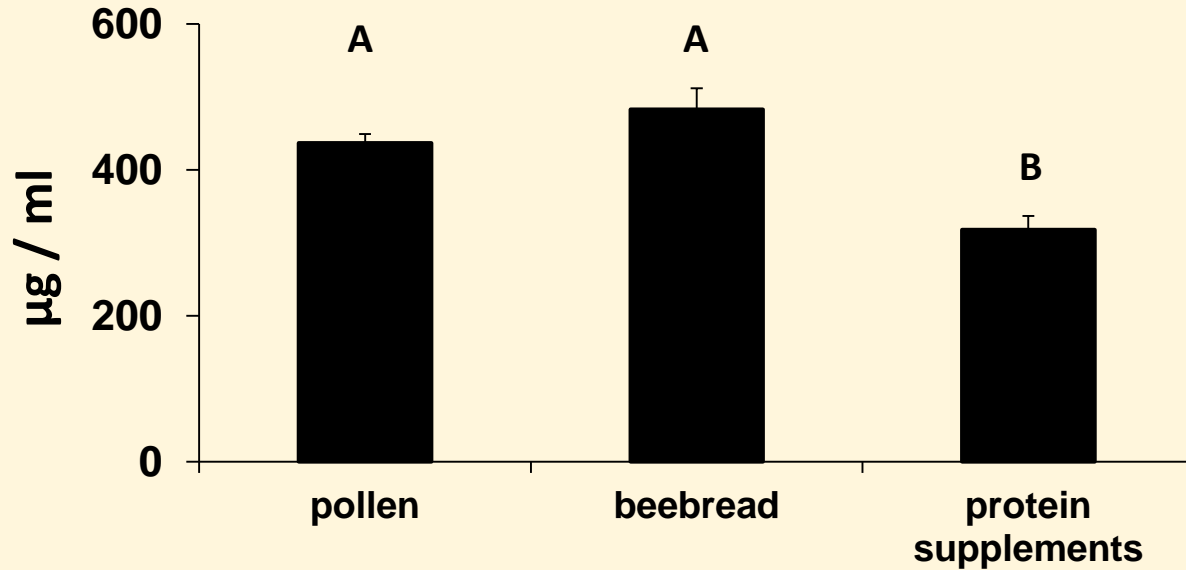
Apiary site-2



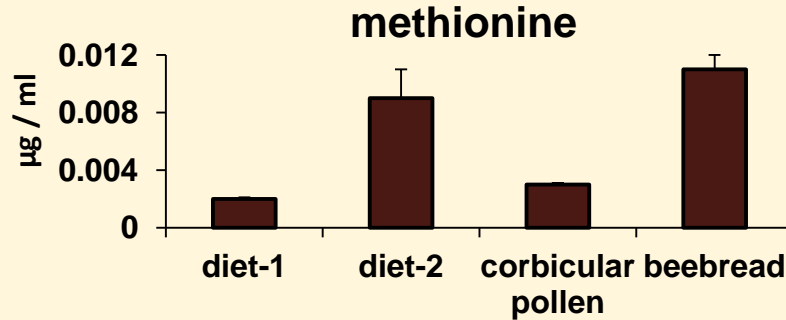
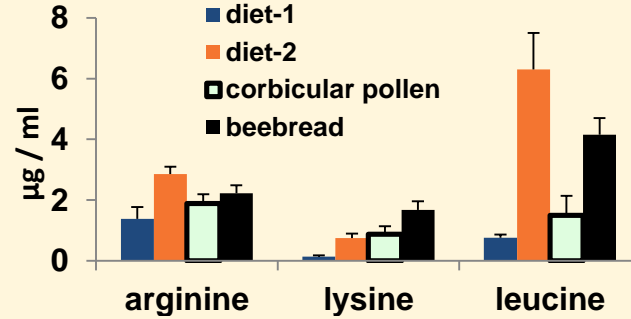
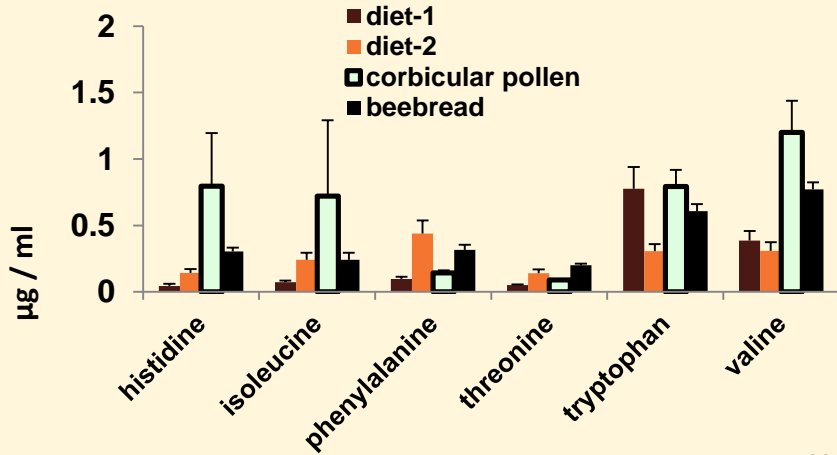
# Logical flow of the project:



# Nutritional Value of Diets vs. Pollen

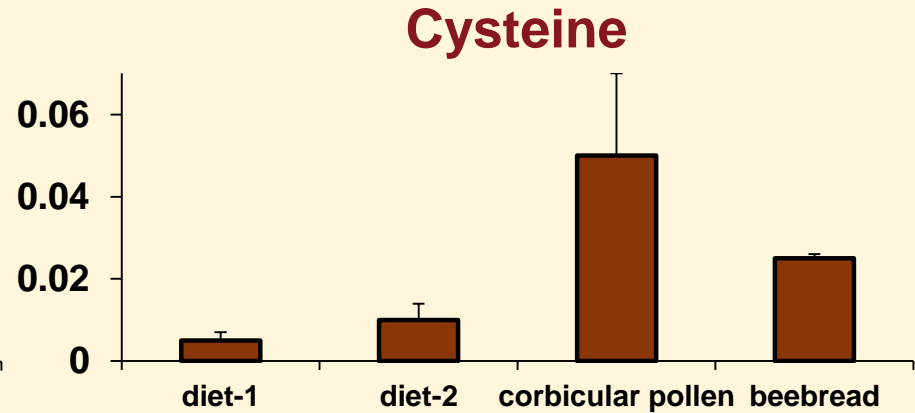
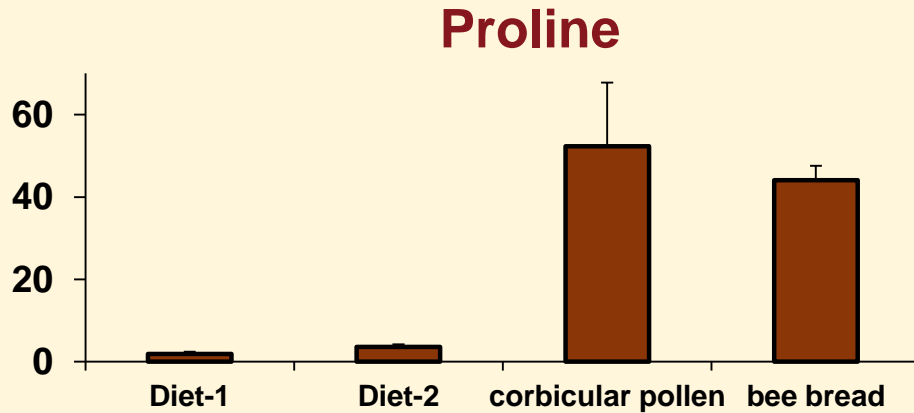


# Essential Amino Acids

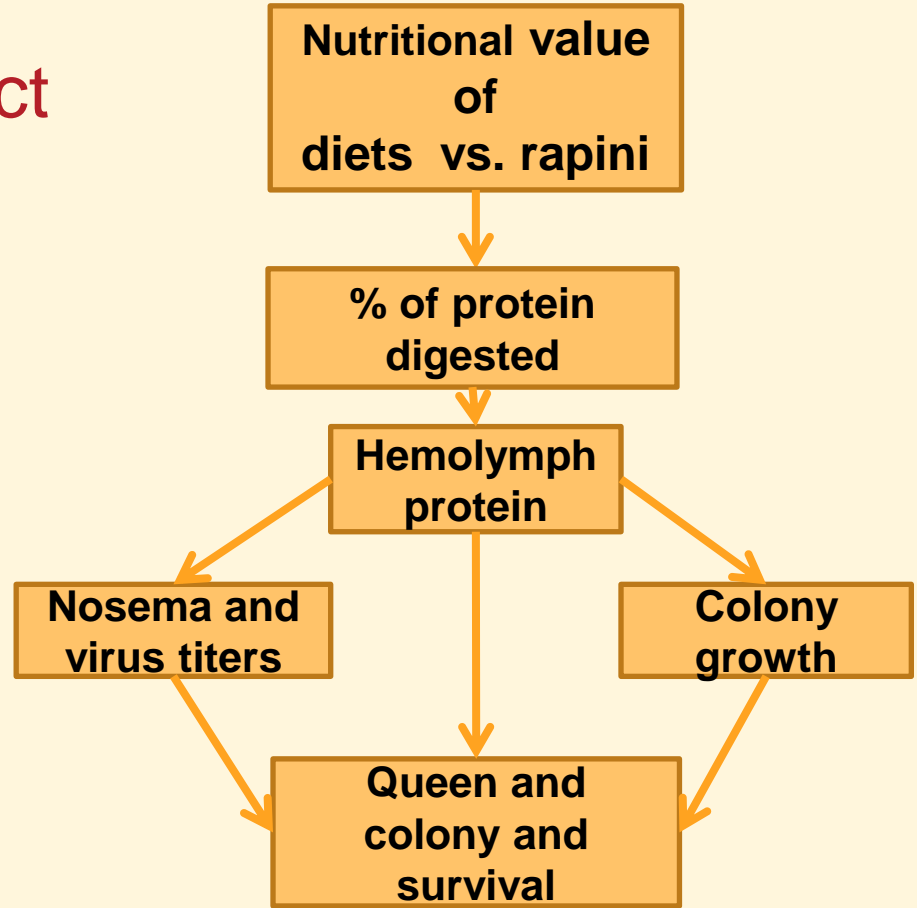


## Conditional Amino Acids: *required during times of physiological stress*

- Proline is used in energy metabolism and in antimicrobial peptides (AMP) such as apidaecin
- Cysteine is required to synthesize glutathione, the cell's major antioxidant; also component of AMP such as royalsin



# Logical flow of the project



# % of Protein Digested

- Analyzed contents of hindgut



Sample nurse bees



Open ventral abdomen



Expose gut contents

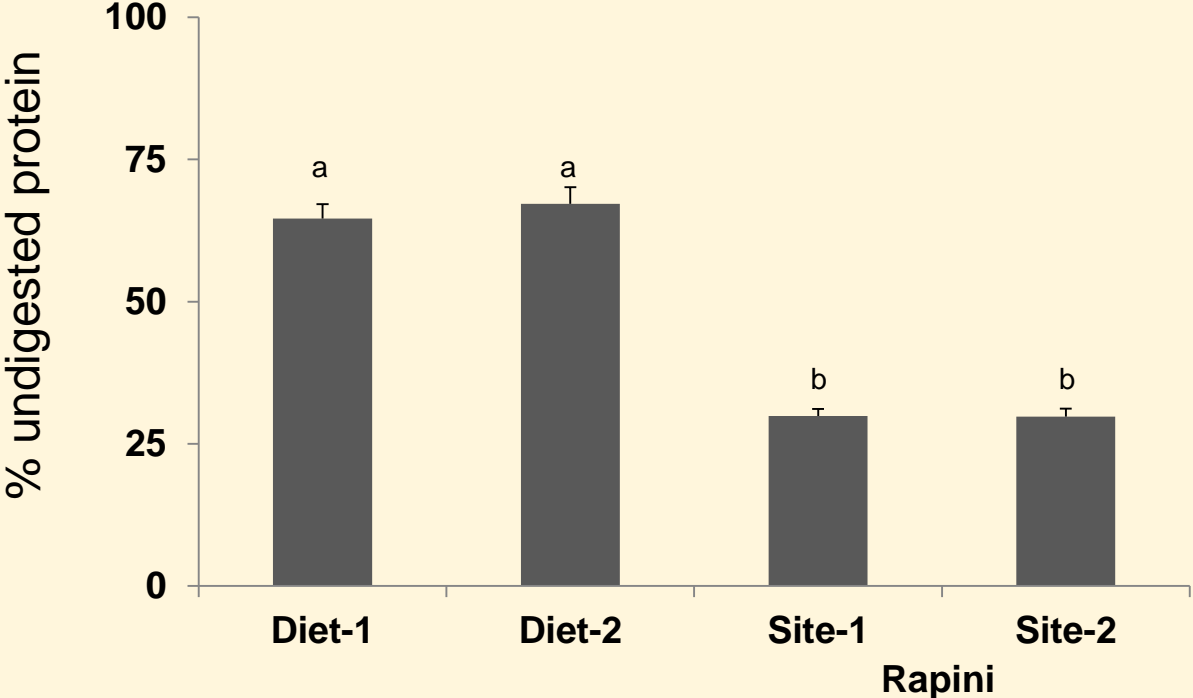


Sample contents



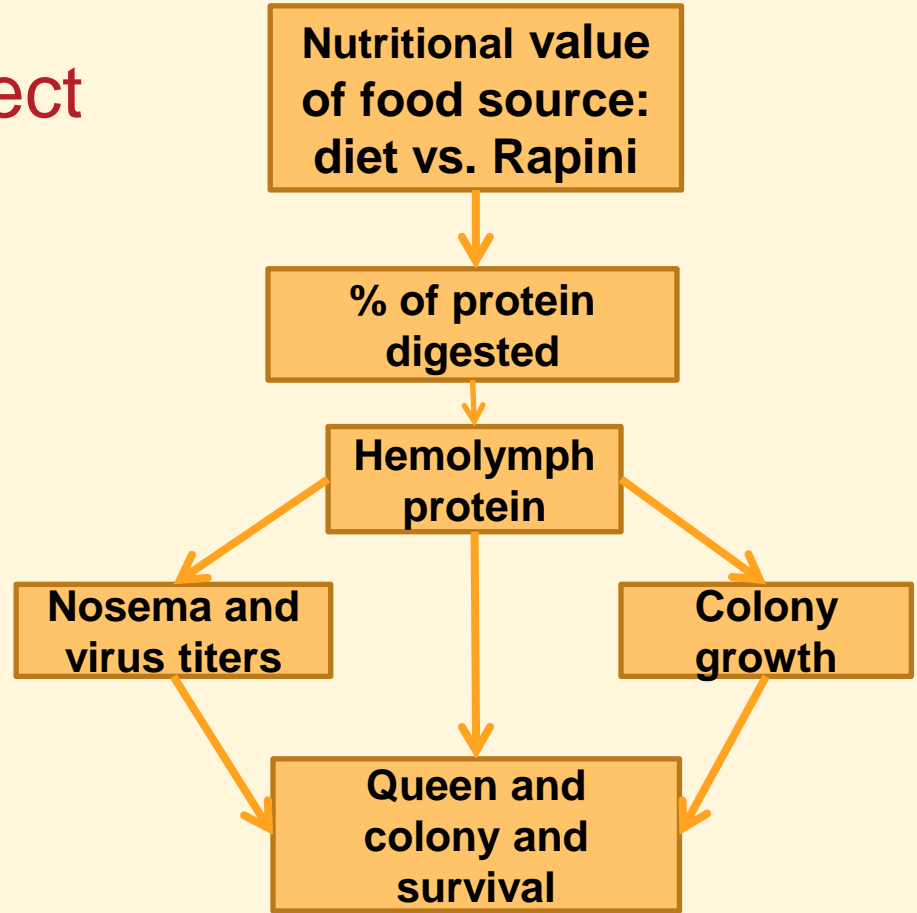
Analyze for protein

# Protein Digestion

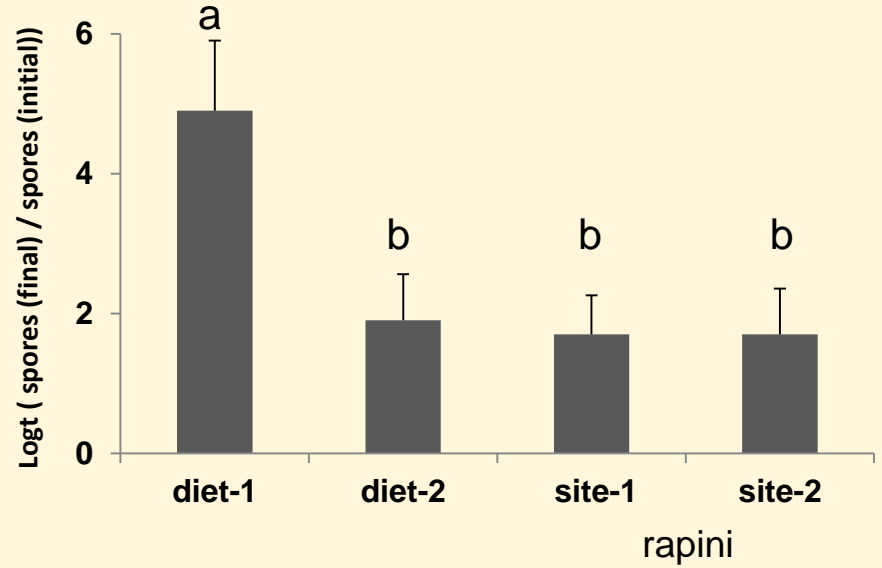
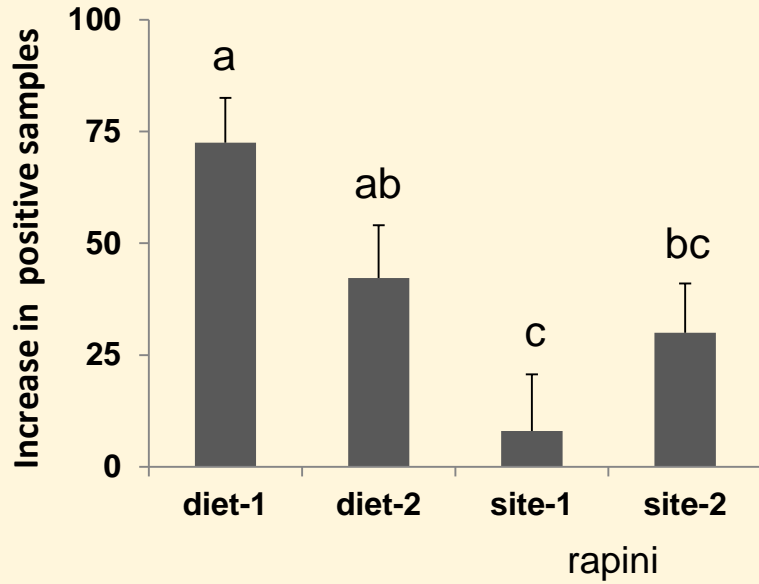




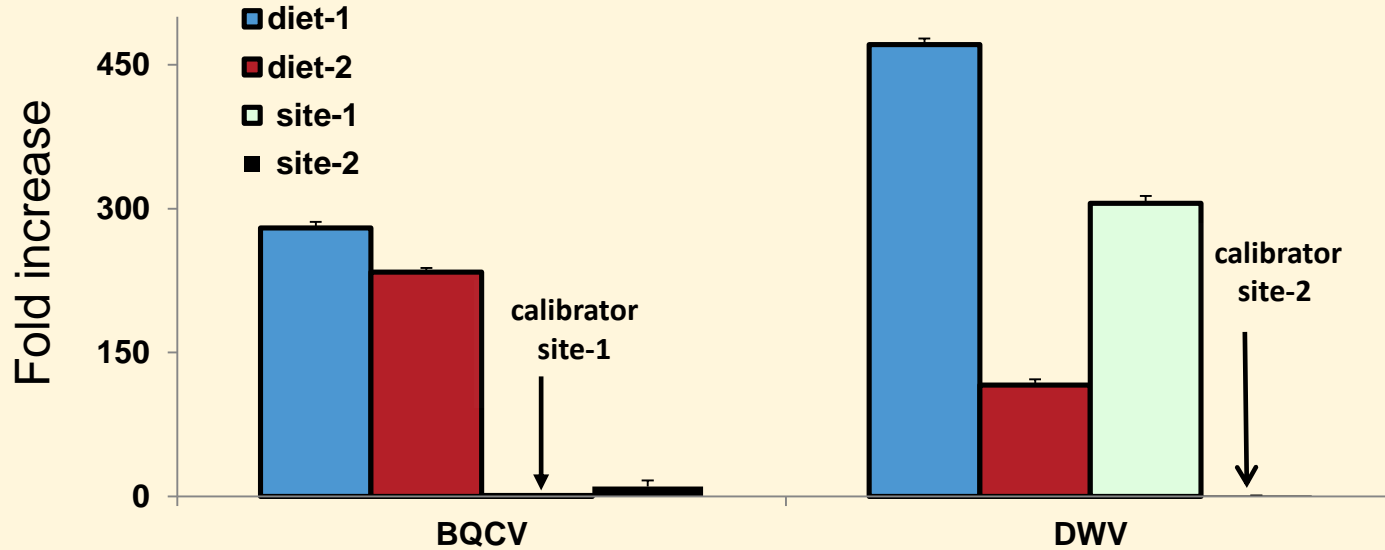
# Logical Flow of the Project



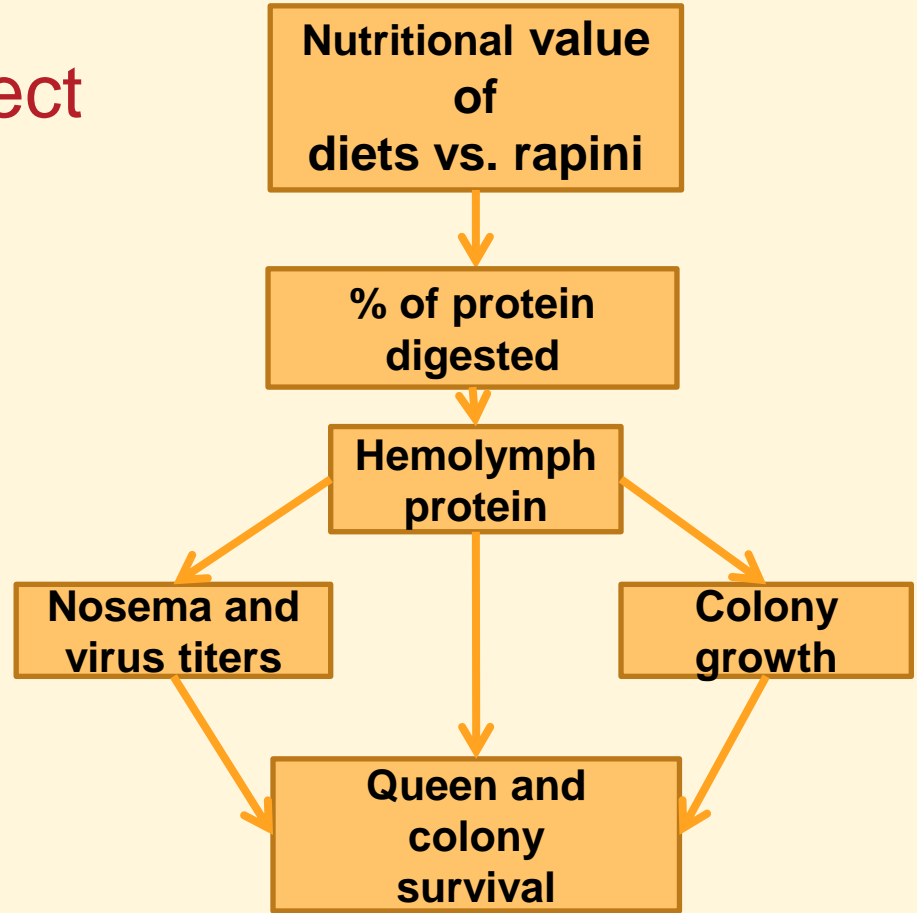
# Nosema Titers



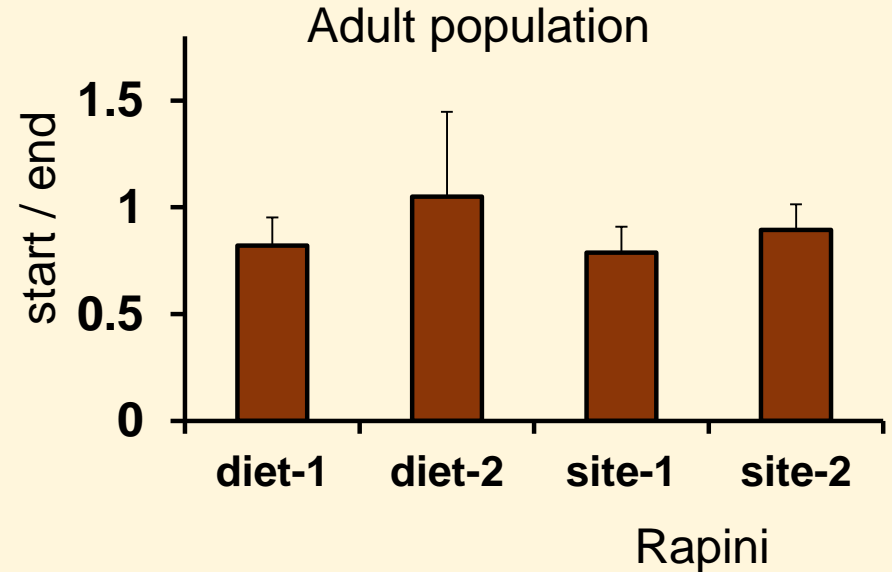
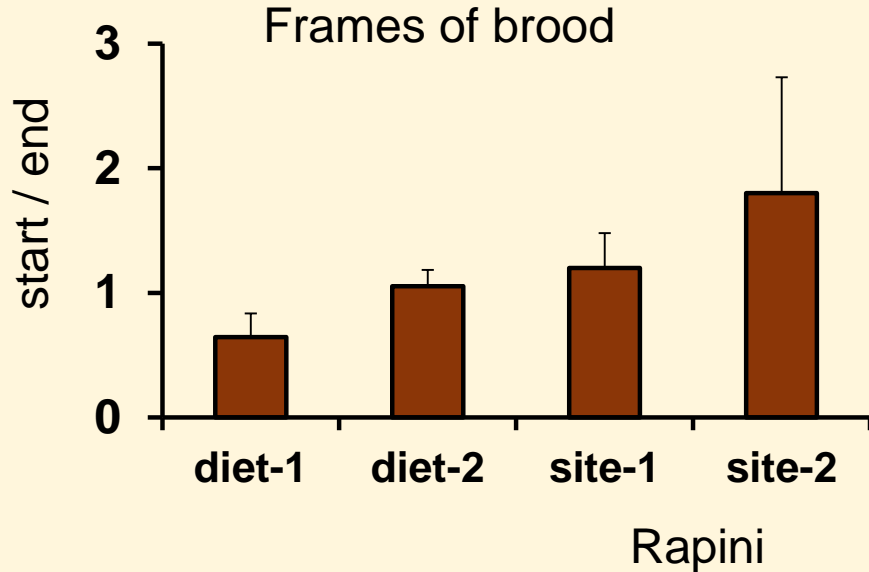
# Virus Titers



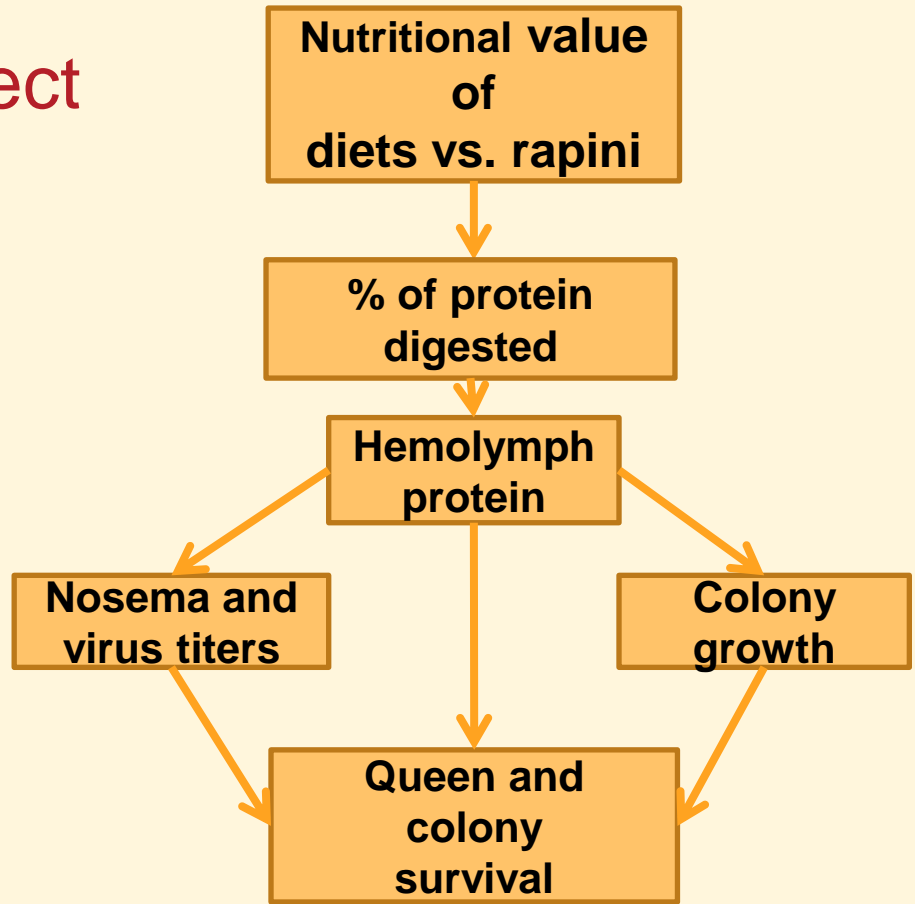
# Logical Flow of the Project



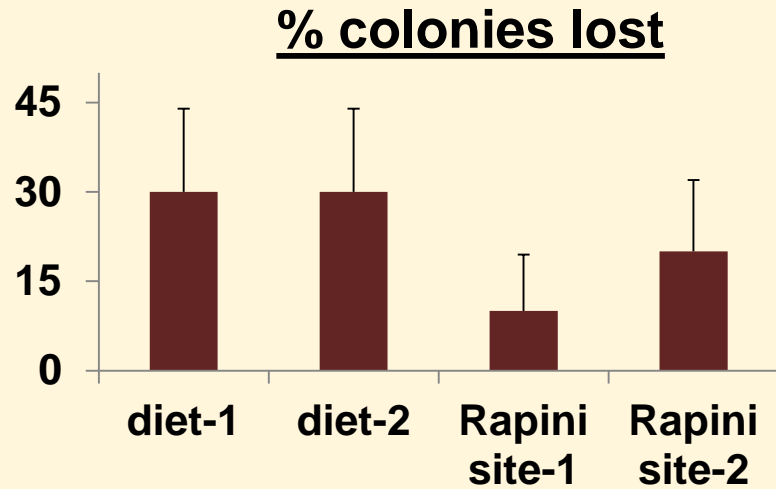
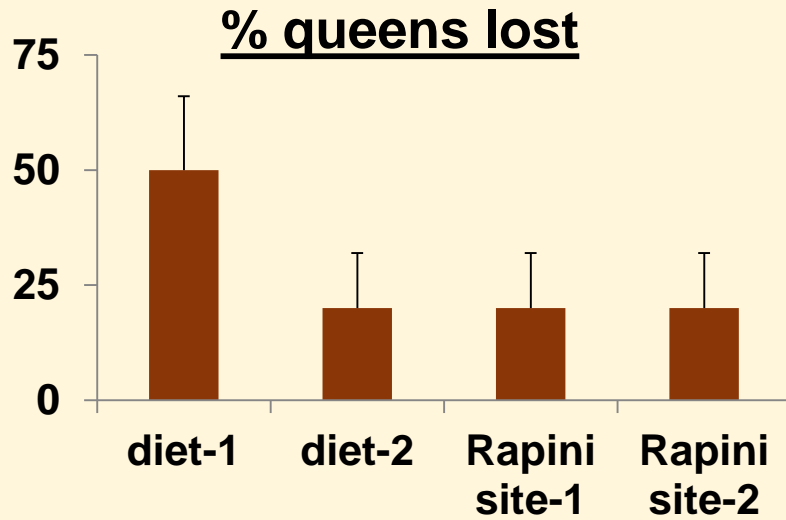
# Brood Production and Population Growth



# Logical Flow of the Project



# Queen and Colony Survival




# Conclusions

- 1) Protein supplements have lower concentrations of protein and certain amino acids than rapini pollen and diets are not digested as well as pollen.**
- 2) Colonies fed protein supplements had higher incidence of disease.**
- 3) Greater queen and colony losses occur with protein supplements than natural forage.**





**Carolyn Breece, Oregon State University**

A close-up photograph of almond blossoms with white petals and pink centers. A semi-transparent circular area on the left side of the image contains text. The background is a soft, light green color.

## **Assessing the Value of Supplemental Forage for Honey Bees during Almond Pollination**

Ramesh Sagili and Carolyn Breece  
Oregon State University  
Honey Bee Lab

# The Issue:

Before and after bloom, almond orchards become “resource deserts”



- Low diversity in pollen and nectar resources
- Poor nutrition
- Low immunity to pests and disease, specifically *Nosema*

# The Solution:

## Plant supplemental forage!

- Project Apis m.: “Seeds for Bees”
- Forage benefits the almond grower and the beekeeper
- Adds diversity to honey bee diet
- Preliminary data: Multi-source pollen = higher protein in HPGs, higher levels of enzymes associated with honey bee immunity



Photo: Project Apis m.

# How will Additional Forage Affect Honey Bees in the Long Term?

Our objective:

To evaluate the effects of supplemental forage prior to and after almond bloom on honey bee nutrition, colony growth, immune system and survival.



# The Plan



3 almond orchards without supplemental bee forage,

16 hives in each orchard



3 almond orchards with supplemental bee forage,

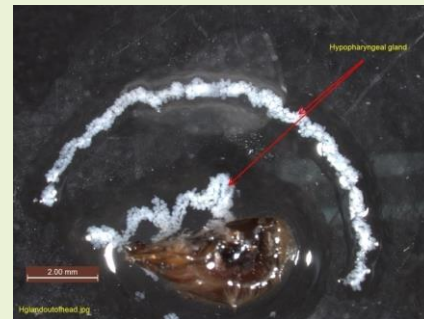
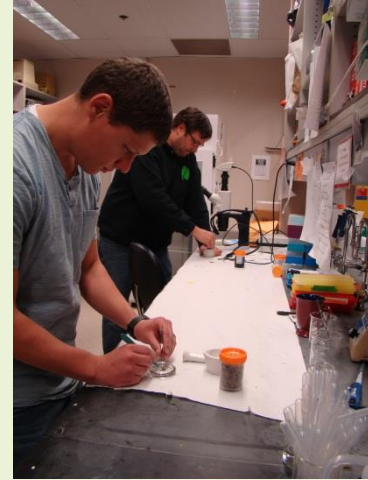
16 hives in each orchard



- We will regularly collect bee samples from hives for nutritional analysis
- We will monitor experimental hives over the entire year for colony strength and survival

# Parameters

- Hypopharyngeal gland protein and lipid analysis
  - *Will honey bees raise healthier young?*
- Immunocompetence
  - *Will honey bees have a stronger immune system?*
- Midgut enzyme activity
  - *Will honey bees digest proteins better?*
- Pest and pathogen analysis
  - *Will better nutrition lead to lower Varroa mites and Nosema levels?*
- Colony growth measurement
  - *How will the whole colony grow over time?*



# Thank you

## Our collaborators

- Dr. Neal Williams, U.C. Davis
- Project Apis m.
- Beekeepers from California and Oregon

We thank Almond Board of California for providing funds for this project.



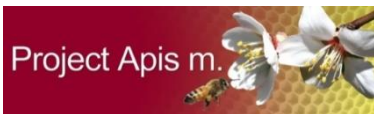


# Neal Williams, UC Davis



# Forage and Integrated Almond Pollination

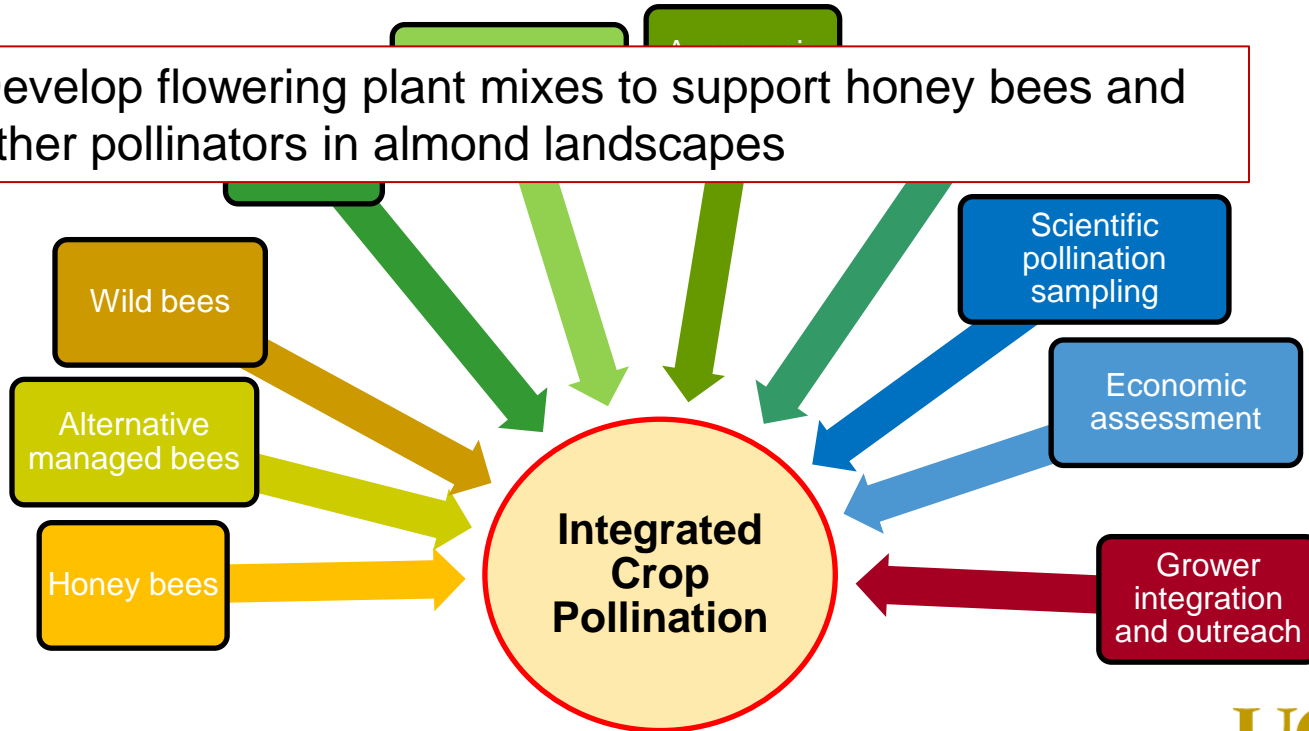
Neal M. Williams  
University of California, Davis



# Integrated Crop Pollination



Develop flowering plant mixes to support honey bees and other pollinators in almond landscapes



## Project Timeline

2013-14 Test mixes different in-orchard locations

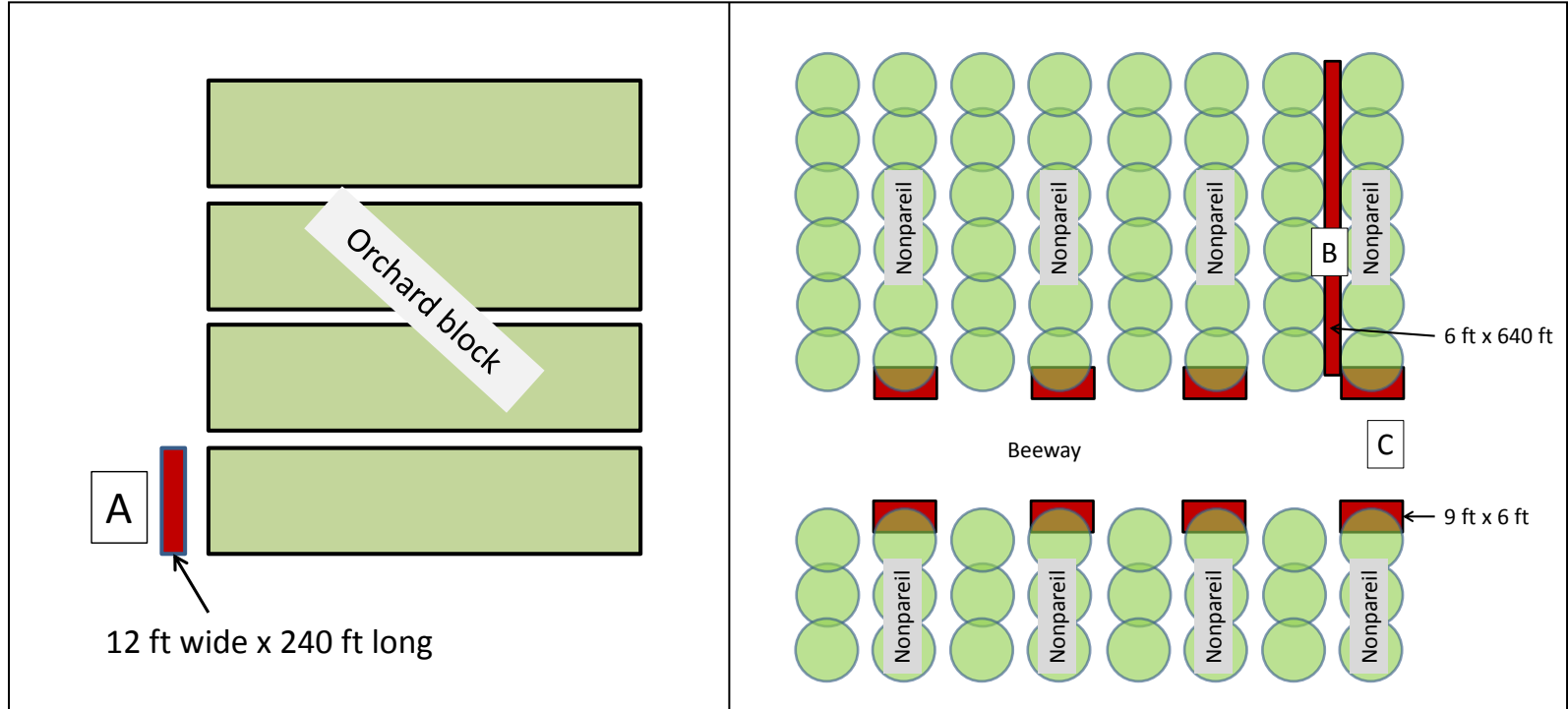
- Honeybee and native bee use of different plant species
- Timing of bee visits relative to mixes relative to almond bloom
- Seasonal and within Day
- Potential competition for pollination with orchard

ABC funded

2014-16 Function impact on bees and pollination

- Examine impact of mix honey bee use, managed blue orchard bee performance

# Testing wildflower plantings in different locations within orchard



# Mix Compositions

- Almond wildflower mix



Great valley phacelia



California blue bell



Five spot



Baby blue eyes



Chinese houses



California poppy

Border plantings only

- Mustard Mix



Rapini mustard

Braco White Mustard

Nemfix Mustard

Radish

- Clover Mix



Crimson Clover

Hykon Rose Clover

Nitro Persian Clover

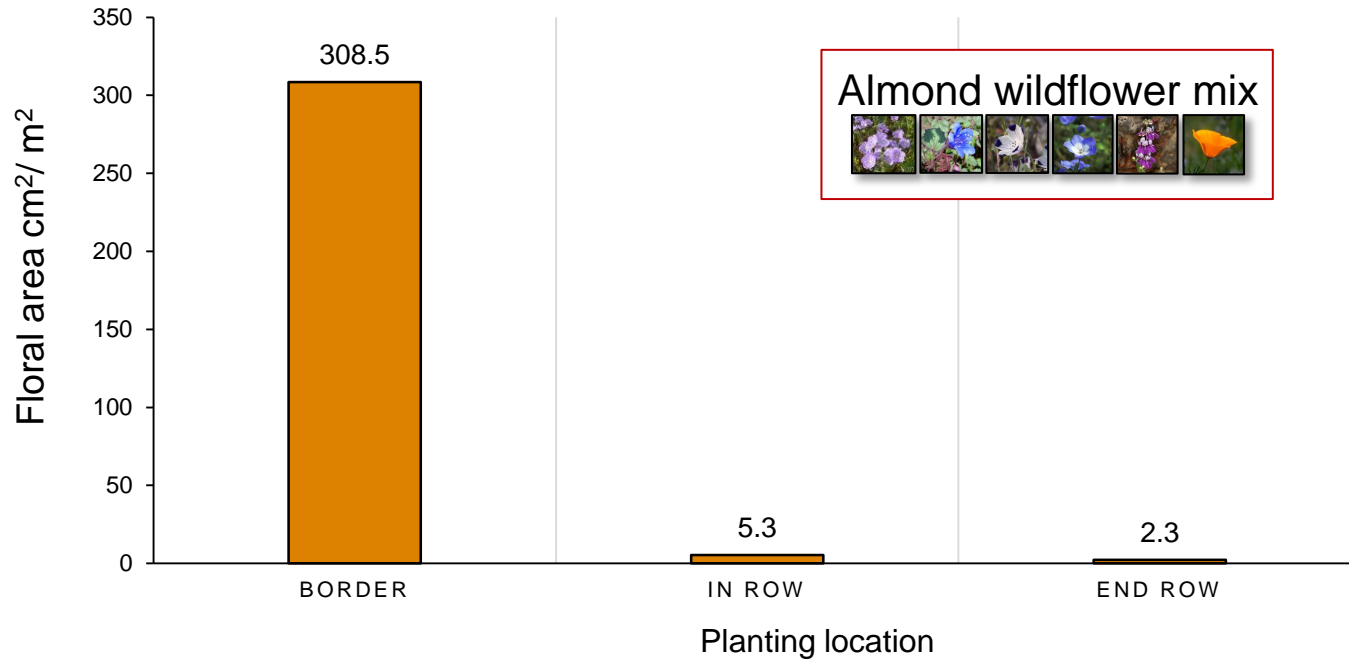
Frontier Balansa Clover

Alyssum

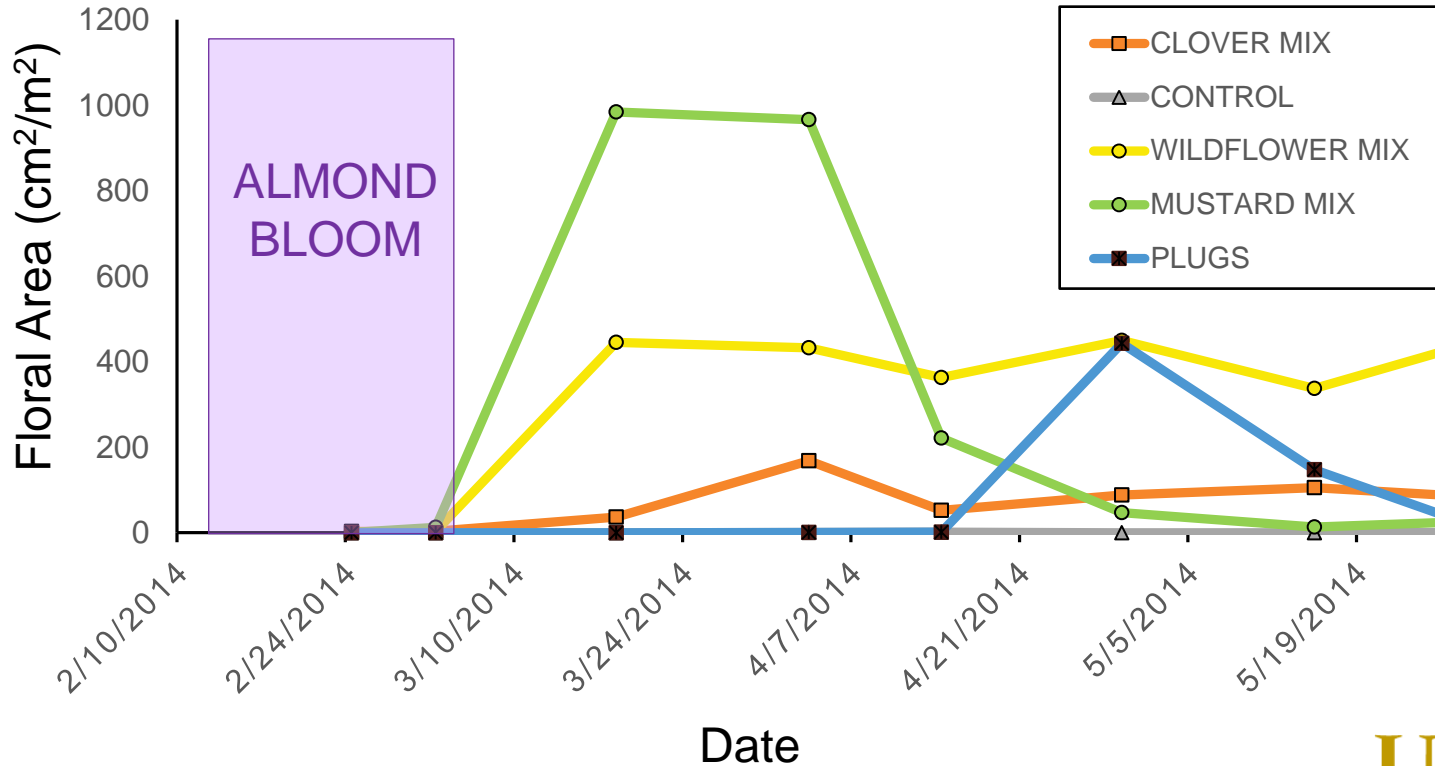
Project Apis m.



# Establishment and Flowering Success

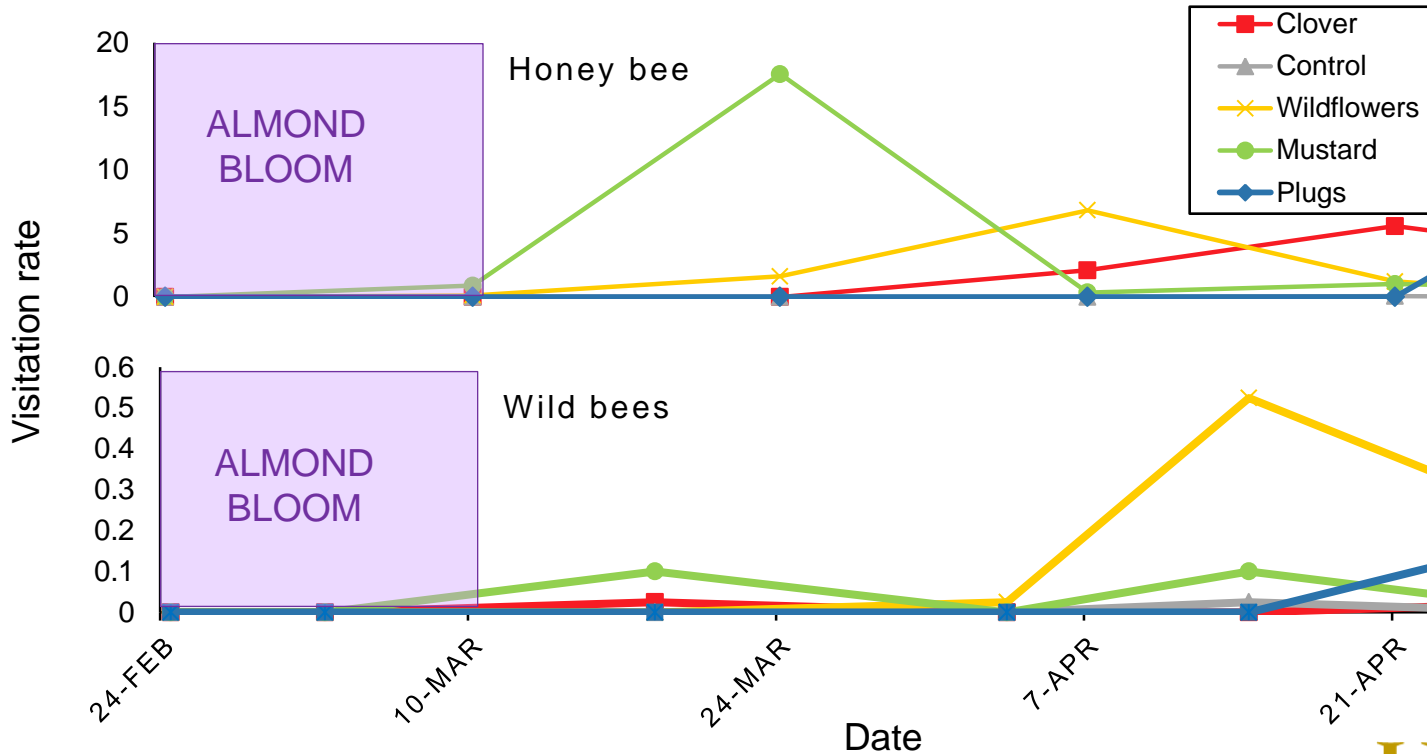


# Bloom Timing

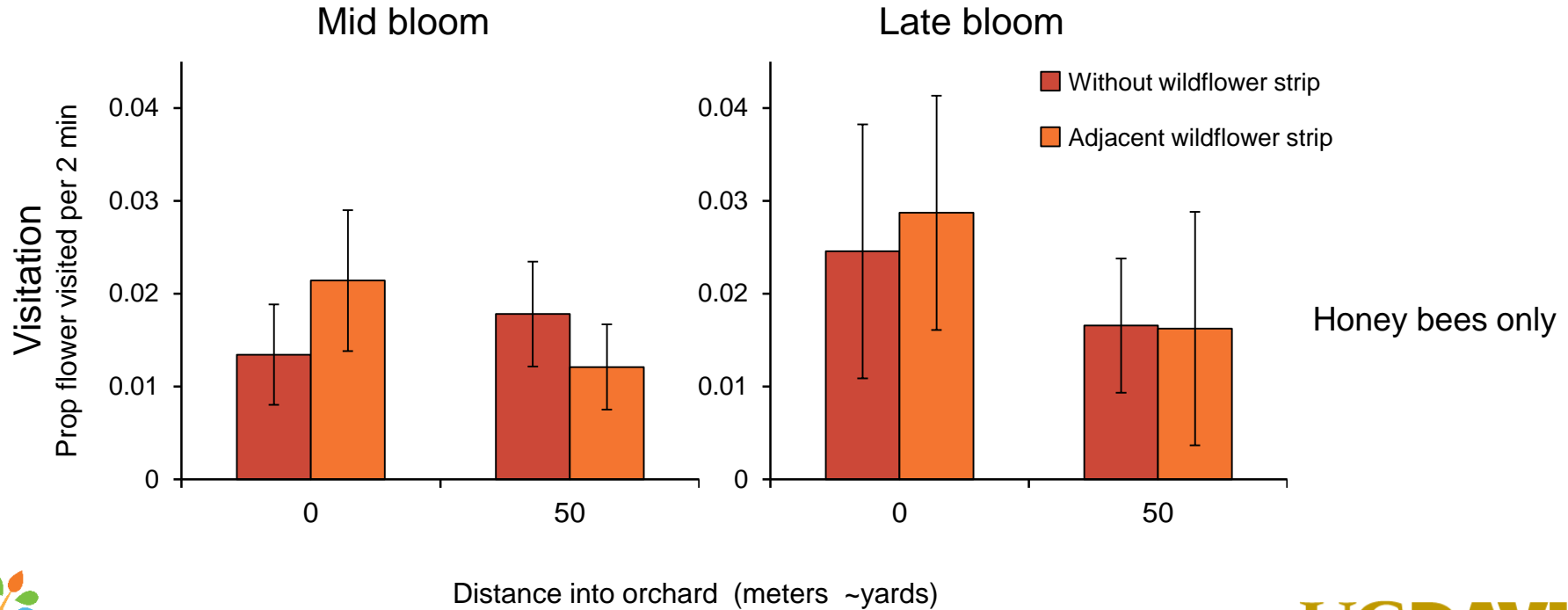




# Bee Visitation to Flower Mixes



# Visitation to Almonds (potential competition of flower strips)



## Summary

- Only the border planting established well, within orchard establishment was poor in the mature orchard
- **Mustard and wildflower** mixes provided the **most bloom** and wildflower flowering persisted longer after almond flowering
- **Mustard** mix, then wildflower and clover mix **attracted the most honeybees**
- **Wildflower** mix, then mustard **attracted the most wild bees**
- Mixes **did not** appear to attract honey bees away from the orchard flowers
- **HOWEVER**, flowering time of mixes was delayed in 2013





**Dennis vanEngelsdorp, University of Maryland**

# Varroa Resistance Monitoring

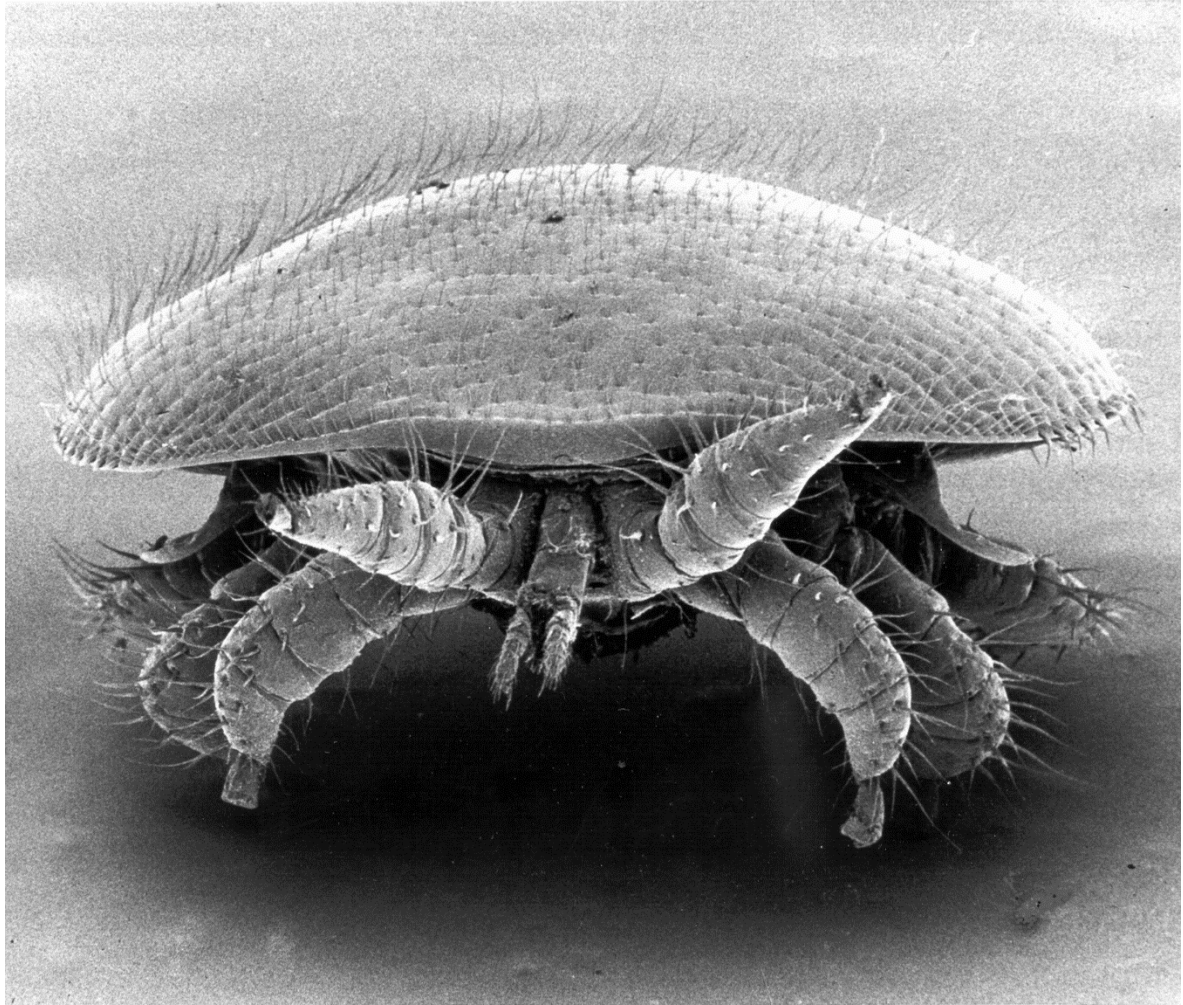
Dennis vanEngelsdorp  
University of Maryland

















**Your Newest All Natural  
Varroa Mite Treatment**

sales@bcwagric.co.uk • 01630 655 722



**MAQS**

**Beehive Strip**  
**healthy bees. healthy planet.**

**For Control of Varroa Mites and Small Hive  
Beetles in Honeybee Colonies**

**KEEP OUT OF REACH OF CHILDREN  
CAUTION**

**ACTIVE INGREDIENTS:**  
Clothianthrin 0.1% (a.i.)  
Bacillus thuringiensis 0.1% (a.i.)  
Other ingredients

**Net Weight:** 10g (0.35 oz)

**Manufacturer:** B.C.W. Beehive Care Warehouse  
100, The Beehive Centre  
Widmore, Wiltshire, BA1 2JG, UK  
Tel: 01630 655 722

**FIRST AID**  
Contains organophosphates that inhibit cholinesterase.

**If on skin or clothing:**  
- Wash skin immediately with plenty of water for 15 minutes.  
- Do not use solvents or detergents for treatment unless advised.

**If in eyes:**  
- Hold eyes open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses if present, after the first 5 minutes, then continue rinsing eye.

**If swallowed:**  
- Call poison control center or doctor for treatment advice.  
- Call poison control center or doctor immediately for treatment advice.  
- Have person sip a glass of water if able to swallow.  
- Do not induce vomiting unless instructed to do so by the poison control center or doctor.  
- Do not give anything by mouth to an unconscious or unresponsive person.

**Note to Physicians:** This product contains cholinesterase inhibitors. It contains an insecticide residue and should not be administered to organophosphate sensitive patients.

**HOTLINE NUMBER:** Have the product container or label with you when calling a poison control center or doctor, or going to a hospital. For emergency medical treatment call 0800 400 009. The customer service or the nearest poison information, including the MSDS, call 1-800-888-3766.

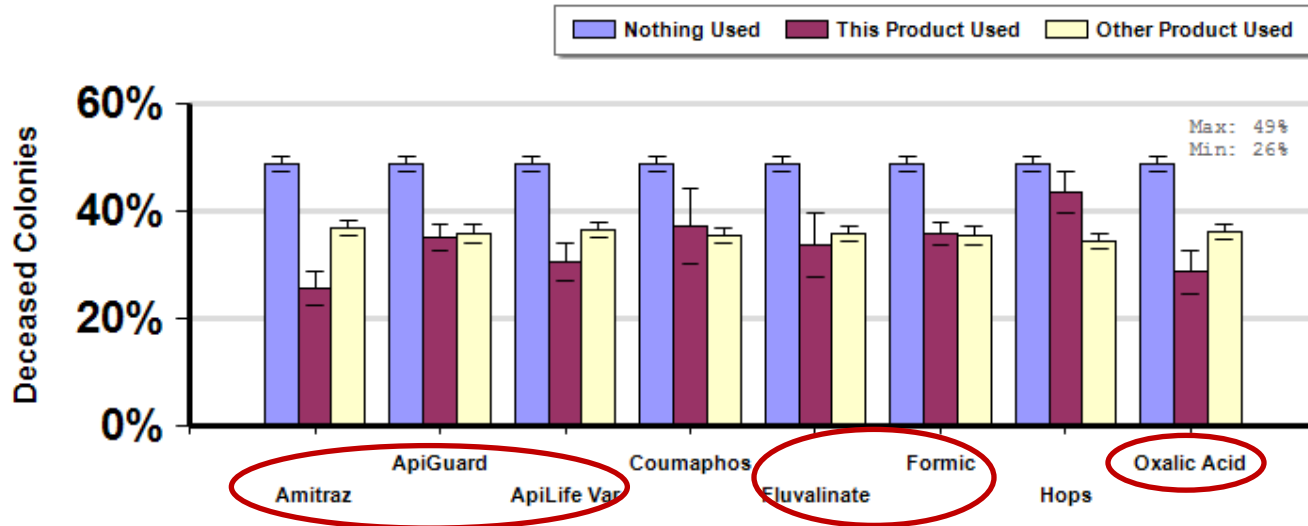




## Varroa Mite Control Product Use By Product

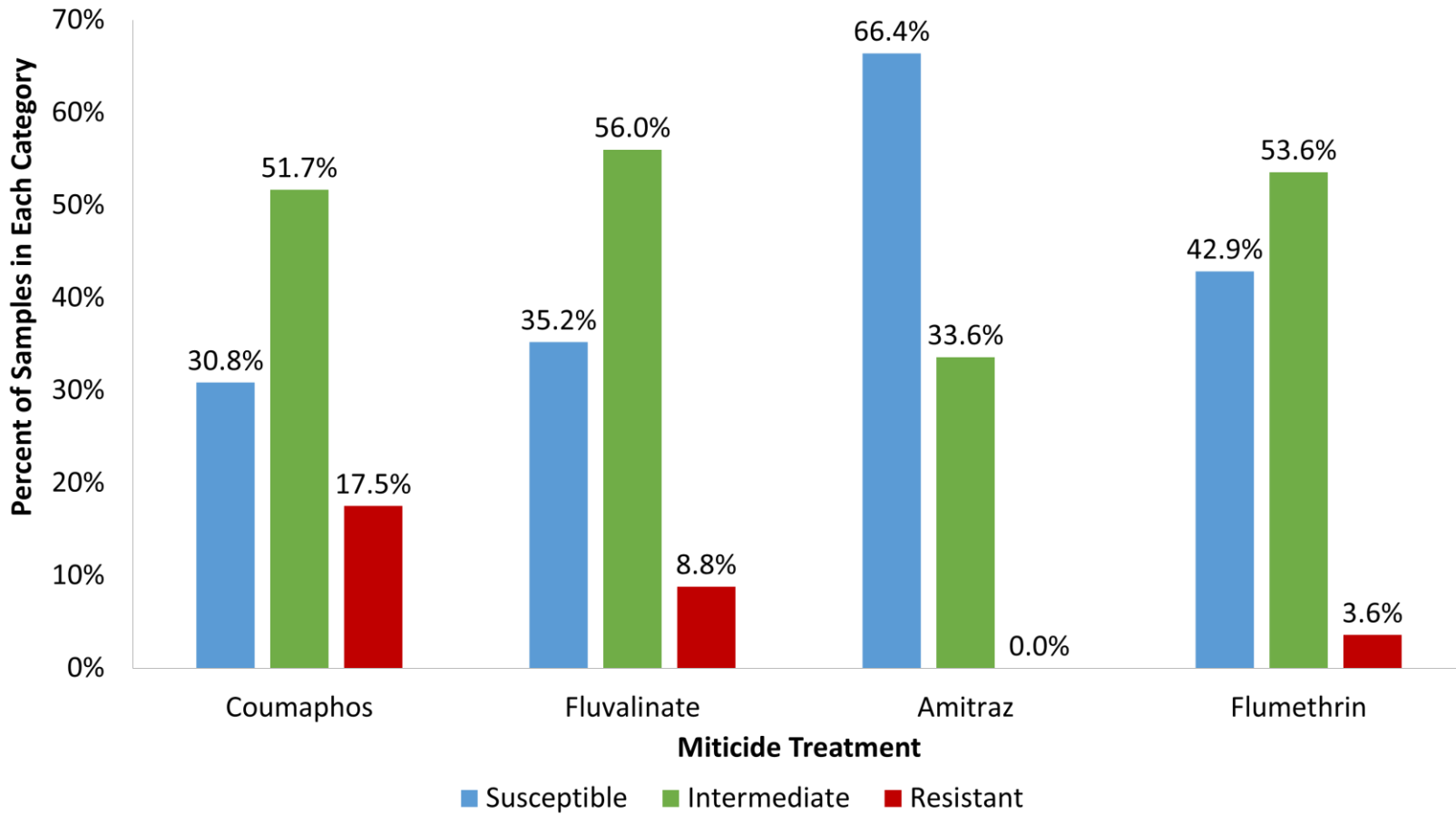
A comparison of average winter colony mortality among beekeepers who reportedly applied different known Varroa control products, at least once, to a majority of their colonies between April and March of the following year. Known Varroa control products include ApiGuard, ApiLife Var, Amitraz, Coumaphos (i.e. CheckMite), Fenpyroximate (Hivastan), Fluvalinate (i.e. Apistan), Formic Acid (i.e. Mite Away II) Sucroside, and Oxalic Acid.

### Some Significant Differences





## National Breakdown between Resistant, Intermediate and Susceptible Varroa (2012-2014)





UNIVERSITY OF  
MARYLAND





**Troy Anderson, Virginia Tech**

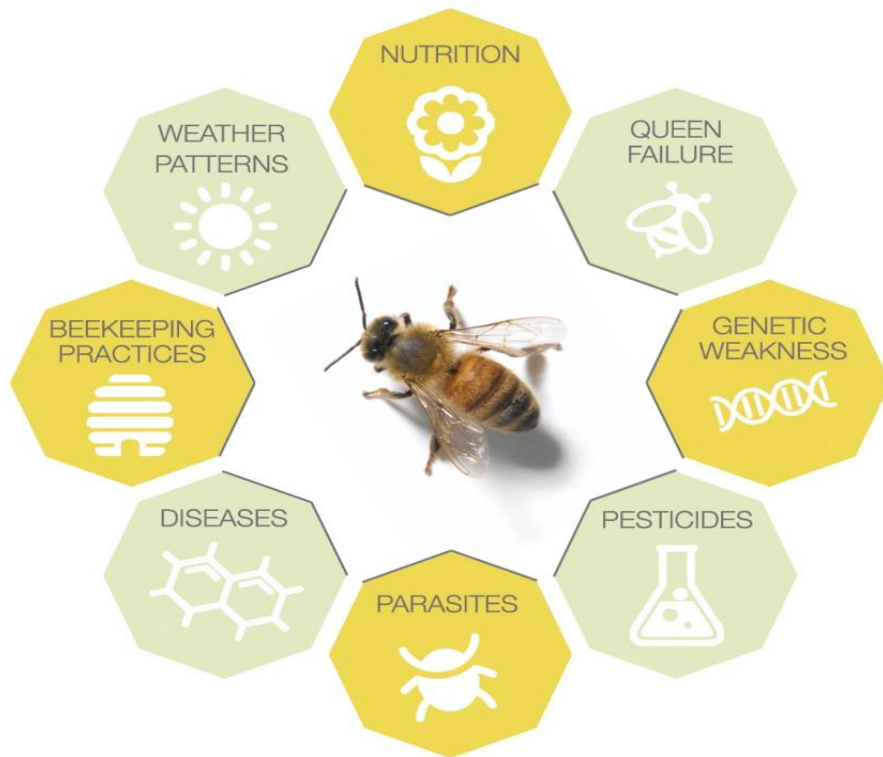
# New Chemistries for Varroa Mite Management

Troy D. Anderson, Ph.D.

Department of Entomology & Fralin  
Life Science Institute, Virginia Tech



# Honey Bee Health: Multiple Stressors, Multiple Interactions



<https://www.bayercropscience.us/our-commitment/bee-health/bee-health-stressors>

# Honey Bee Health: Pesticide Risk Characterization

Exposure

Fate, Persistence, & Application

X

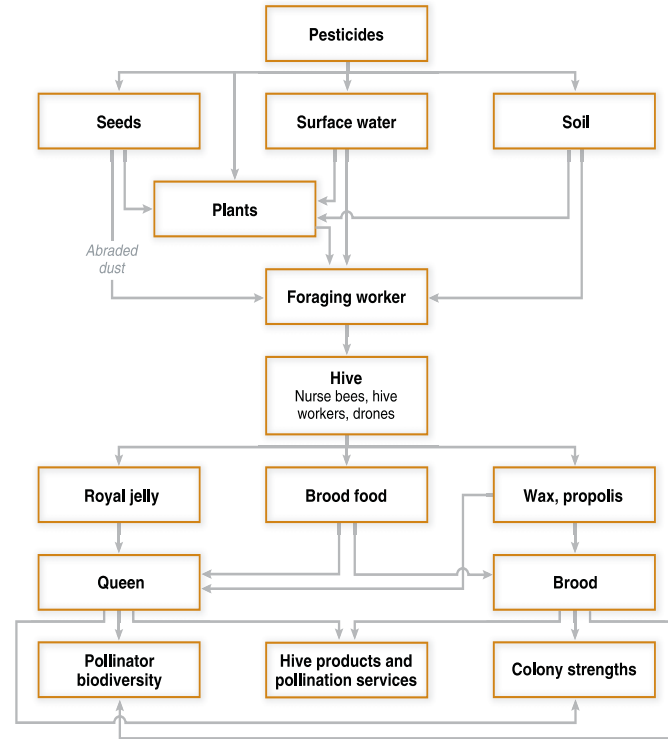
Toxicity

Laboratory vs. Field Testing

X

Risk

Predict Effects of Pesticide  
Use, Misuse, & Safety



Fairbrother et al. 2014

# Pest Management Challenge: Varroa Mite

Hematophagous Mite

~30% Bee Colony Losses

Infectious Disease Vector

Limited Chemical Control  
Strategies for Beekeepers



# Pest Management Challenge: Standard In-Hive Acaricides

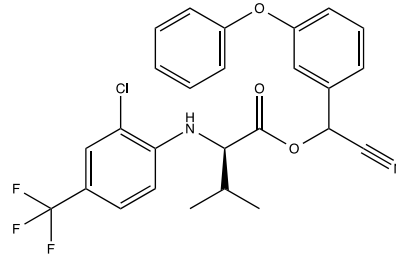
Widespread Target-Site and  
Metabolic Resistance

Increase Mixture Toxicity  
(Williams and Anderson 2013)

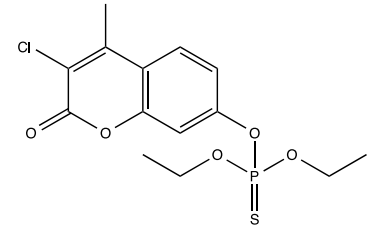
Impair Bee Reproduction  
(Burley et al. 2009)

Reduce Bee Nutrition and  
Immunity (Reeves et al. 2014)

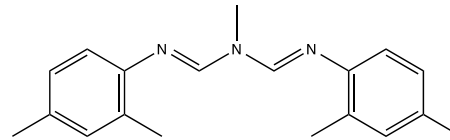
Increase Pathogen Infection  
(Reeves et al. 2014)



*tau*-Fluvalinate  
(Apistan<sup>®</sup>, 10.0% ai)



Coumaphos  
(CheckMite+<sup>™</sup>, 10.3% ai)



Amitraz  
(Apivar<sup>®</sup>, 3.3% ai)

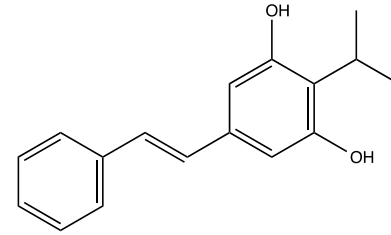
# Pest Management Challenge: Alternative In-Hive Acaricides

Natural Stilbenoid Isolate in *Photorhabdus* Bacteria of *Heterorhabditis* Nematodes

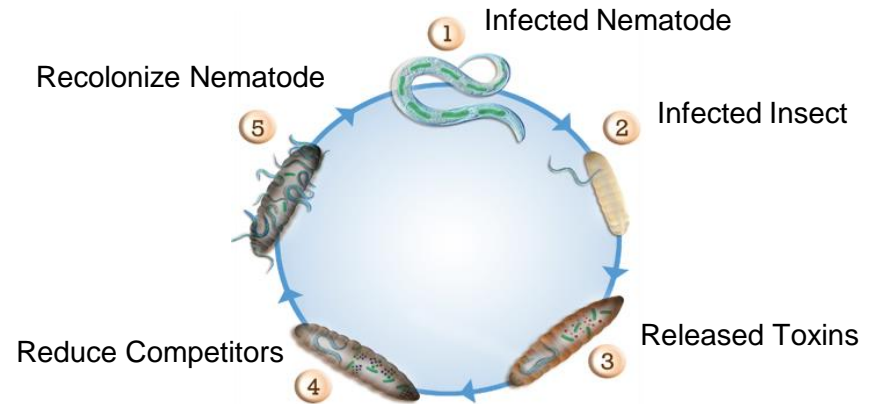
Pesticide Activity Against Nematodes and Insects (Boina et al. 2008, Boina and Bloomquist 2009)

Inhibits Growth, Decreases Survival, and Reduces Cl<sup>-</sup> Uptake (Boina and Bloomquist 2009)

Voltage-Gated Cl<sup>-</sup> Channel Blocker (Jenson et al. 2013)



3,5-dihydroxy-4-isopropylstilbene



# Pest Management Challenge: Alternative In-Hive Acaricides

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Voltage-Gated Cl<sup>-</sup> Channel Blocker  
(Jenson et al. 2013)

## Voltage-gated (VGCC)

- Found in plasma and intracellular organelle membranes
- Involved in many cell functions including volume regulation and stabilizing membrane potentials of excitable tissues; however, not much is known about their functionality in insects

## Ligand-gated (LGCC)

- Activated by an assortment of neurotransmitters such as  $\gamma$ -aminobutyric acid (GABA), glutamate, and histamine
- Pentameric formation of subunits that span the membrane with an intrinsic chloride channel associated with it
- When the neurotransmitter binds to the ligand-gated channel, it activates the chloride channel allowing Cl<sup>-</sup> ions to flow in, which in turn hyperpolarizes the membrane potential

## Calcium- activated (CACC)

- More recently been found in nematodes and insects, with the least amount of information available for this class



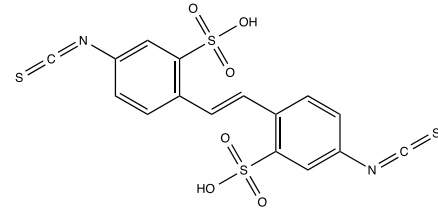
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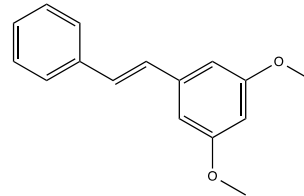
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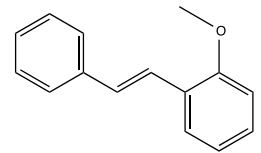
Voltage-Gated Cl<sup>-</sup> Channel Blocker  
(Jenson et al. 2013)



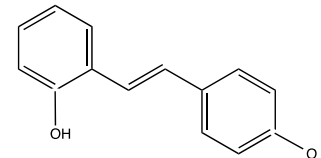
4, 4'-diisothiocyanatostilbene-2,  
2'-disulfonic acid (10.0% ai)



3,5-dimethoxystilbene (10.0% ai)



2-methoxystilbene (10.0% ai)



(E)-2-(4-methoxystyryl)phenol (10.0% ai)

# Field Efficacy Testing: Standard vs. Alternative Acaricides



Philene Vu, MS Student



Price's Fork, Kentland Farm, and Moore Farm Apiaries in Blacksburg, VA



Sample Varroa Mites from Brood Frames in Each Bee Colony



Collect ~300 Brood-Nest Bees from Each Frame for Acaricide Bioassays



Expose Varroa Mites to Acaricide-Treated Tabs for 3- and 6-hr Intervals



Rinse Brood-Nest Bees with Ethanol to Remove Remaining Varroa Mites



Mites are exposed to acaricide resulting in paralysis

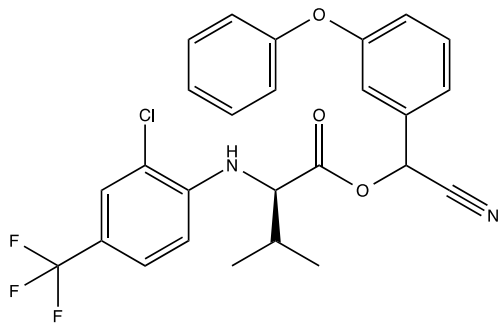


Bees walk on acaricide strips and pick up molecules



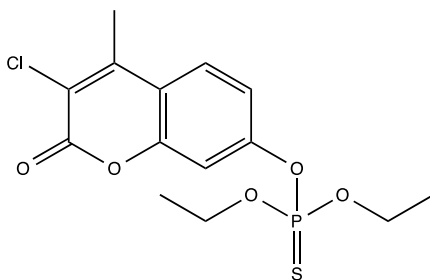
Bees distribute acaricide via contact with each other

# Field Efficacy Testing: Standard vs. Alternative Acaricides



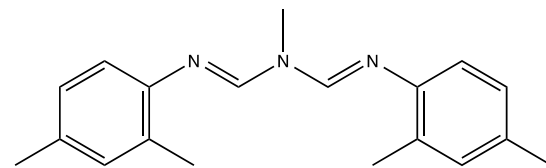
*tau*-Fluvalinate  
(Apistan<sup>®</sup>, 10.0% ai)

37% - 45% Efficacy (6 hr,  $n = 12$ )



Coumaphos  
(CheckMite+<sup>™</sup>, 10.3% ai)

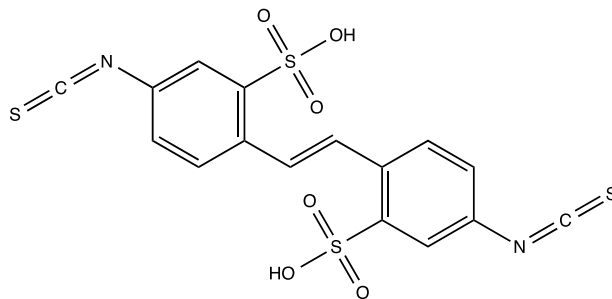
26% - 51% Efficacy (6 hr,  $n = 12$ )



Amitraz  
(Apivar<sup>®</sup>, 3.3% ai)

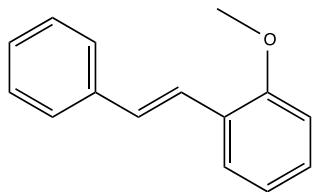
100% Efficacy (6 hr,  $n = 12$ )

# Field Efficacy Testing: Standard vs. Alternative Acaricides

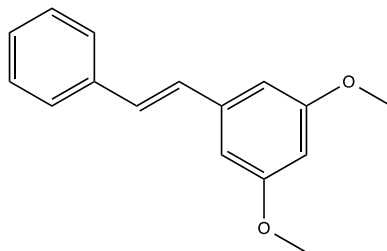


4,4'-diisothiocyanatostilbene-2, 2'-disulfonic acid (10.0% ai)

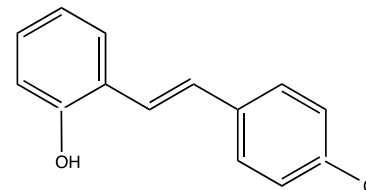
61% - 70% Efficacy (6 hr,  $n = 12$ )



2-methoxystilbene (10.0% ai)



3,5-dimethoxystilbene (10.0% ai)



(E)-2-(4-methoxystyryl)phenol (10.0% ai)

# Future Directions: New Resistance-Breaking Acaricides

Acaricide resistance monitoring and management of physiological mechanisms that confer resistance in Varroa mite populations

Voltage-gated chloride channel can be exploited as a unique target site for new acaricide chemistries to manage Varroa mite populations

Stilbene chemistries with increased field efficacy and resistance-breaking activity against resistant Varroa mite populations

Alternative acaricides to guide the target-site discovery and development of new resistance-breaking chemistries for Varroa mite management

# Acknowledgements

Almond Board of California (Project 14.POLL6A)

Prof. Dennis vanEngelsdorp, University of Maryland

Philene Vu, MS Student, Virginia Tech

Nicholas Larson, PhD Student, Virginia Tech

Ian Sandum, UG Student, Virginia Tech

Dr. Lacey Jenson, Post-Doctoral Fellow, Virginia Tech

