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
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)
ANOVA statistics, $F=8.1816; df=30,258; P<0.0001$
ANOVA statistics, $F=10.9699; \text{df}=30,258; P<0.0001$
Treatment Timing and Trapping - 2014

NOW Trap Counts, Ripon, 2014

- Male NOW
- NOW Eggs
- NOW DD since Jan 1
- NOW DD since Mar 3
- NOW DD since Biofix, May 2

400 PTB DD = May 4
100 NOW DD = May 12
(based on egg traps)

Spray dates
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

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

John J. Beck,* Bradley S. Higbee,† Douglas M. Light,† Wai S. Gee,* Glory B. Merrill,† and Jennifer M. Hayashi†

†Plant Mycotoxin Research, Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710, United States
‡Paramount Farming Co., 33141 E. Lermo Highway, Bakersfield, California 93308, United States

OH


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

- Male + Female Moths Captured per Trap per Week
  - Conventional Blend
  - Conventional Almond Meal

Graph showing male and female moth captures from April 3 to September 18, with peaks in different trapping periods.
2014 Lost Hills Areawide Project - Mating Disruption Orchard

**Male + Female Moths Captured per Trap per Week**

- Mating Disruption Blend
- Mating Disruption Almond Meal

![Graph showing moth captures over time](image-url)
Graph showing the number of male and female moths captured per trap per week from 4/3 to 9/18. The graph compares Mating Disruption Blend (green triangles) and Mating Disruption Almond Meal (purple crosses). The graph highlights HS Application Decisions with a red arrow.

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 1st year

• Need 2-3 years of data
Pheromone and Host Plant Volatiles for NOW Monitoring
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

Legend:
- Dry Pistachio Mummies
- Wet Pistachio Mummies
- Crushed Pistachio Kernels
- Almond Meal
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!
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.
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.
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

1. Chico
2. Manteca
3. Merced
4. Delhi
5, 6. Gustine
7. Le Grand
8, 9, 10. Lost Hills
11. Lost Hills
12, 13. McKittrick
14. Bakersfield
15, 16. McFarland
17. Bakersfield

L. zonatus
L. clypealis
Distinguishing these two species

_L. clypealis_ and _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**
- L. clypealis: 63%
- L. zonatus: 37%

**Pistachio**
- L. clypealis: 25%
- L. zonatus: 75%

**Pomegranate**
- L. clypealis: 94%
- L. zonatus: 6%

- L. clypealis: Pointed clypeus
- L. zonatus: Two spots
Molecular Identification of Species

**L. clypealis**

- *L. clypealis* on almonds and pistachios are interbreeding, moving between host plants.
- No cryptic species were detected.
Most *L. zonatus* are one genotype

One site had two genotypes

*L. zonatus* were more abundant in Fall

\[ S = \text{Shafter} \]
\[ M = \text{McKittrick} \]
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

Winton

Nonpareil, Fritz

W1
W2
W3
W4
W5
W6
W7
W8

Control
Puncture
Lc or Lz

T1, T2

adunnphotography.com
Results - Total Almond Drop

<table>
<thead>
<tr>
<th></th>
<th>Fritz</th>
<th>NonPar</th>
<th>Monterey</th>
<th>Carmel</th>
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<tr>
<td>Control</td>
<td></td>
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<tr>
<td>Punctured</td>
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<tr>
<td>L. clupealis</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>L. zonatus</td>
<td></td>
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</tr>
</tbody>
</table>

Percent Drop

- Fritz: 30%
- NonPar: 20%
- Monterey: 50%
- Carmel: 40%
Almond Kernel Damage

- Fritz
- NonPar
- Monterey
- Carmel

- Control
- L. clypealis
- L. zonatus

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

Mark Demkovitch¹
Joel Siegel²
May Berenbaum¹

¹ University of Illinois at Urbana-Champaign
² 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

Bifenthrin LC₅₀ in R347

LC₅₀ (ppm)

Generation

0 0.5 1 1.5 2 2.5 3
1 2 3 4 5 6 7 8 9 10 11 12 13
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
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

Rapini

Apiary site-1 and Apiary site-2

Diet-2
Logical flow of the project:

Nutritional value of diets vs. rapini

% of protein digested

Hemolymph protein

Nosema and virus titers

Queen and colony survival

Colony growth
Nutritional Value of Diets vs. Pollen

![Bar chart showing the nutritional value of different diets and pollen.]

- Pollen: 400 µg/ml
- Beebread: 600 µg/ml
- Protein supplements: 200 µg/ml
Essential Amino Acids

- histidine
- isoleucine
- phenylalanine
- threonine
- tryptophan
- valine

- arginine
- lysine
- leucine

- methionine
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.

### Proline

<table>
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<th>Diet</th>
<th>Corbicular Pollen</th>
<th>Bee Bread</th>
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<tbody>
<tr>
<td>Diet-1</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Diet-2</td>
<td>High</td>
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</table>

### Cysteine

<table>
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<th>Diet</th>
<th>Corbicular Pollen</th>
<th>Bee Bread</th>
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<tr>
<td>Diet-1</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Diet-2</td>
<td>High</td>
<td>High</td>
</tr>
</tbody>
</table>
Logical flow of the project

Nutritional value of diets vs. rapini

% of protein digested

Hemolymph protein

Nosema and virus titers

Colony growth

Queen and colony and survival
% of Protein Digested

- Analyzed contents of hindgut

Sample nurse bees

- Open ventral abdomen
- Expose gut contents
- Sample contents
- Analyze for protein
Protein Digestion

% undigested protein

- Diet-1: a
- Diet-2: a
- Site-1: b
- Site-2: b

Rapini
Logical Flow of the Project

Nutritional value of food source: diet vs. Rapini

% of protein digested

Hemolymph protein

Nosema and virus titers

Colony growth

Queen and colony and survival
Nosema Titers

Increase in positive samples

- diet-1: a
- diet-2: ab
- site-1: c
- site-2: bc

Logt (spores (final) / spores (initial))

- diet-1: a
- diet-2: b
- site-1: b
- site-2: b

rapini
Virus Titers

- BQCV
  - diet-1
  - site-1
  - calibrator site-1

- DWV
  - diet-2
  - site-2
  - calibrator site-2

Fold increase

Logical Flow of the Project

- Nutritional value of diets vs. rapini
  - % of protein digested
    - Hemolymph protein
      - Nosema and virus titers
      - Colony growth
    - Queen and colony survival
Brood Production and Population Growth

Frames of brood

Rapini

Adult population

Rapini
Logical Flow of the Project

Nutritional value of diets vs. rapini

% of protein digested

Hemolymph protein

Nosema and virus titers

Colony growth

Queen and colony survival
Queen and Colony Survival

% queens lost

% colonies lost

diet-1  | diet-2  | Rapini site-1  | Rapini site-2

0  | 15  | 30  | 45  

site-1  | site-2

% queens lost

% colonies lost

diet-1  | diet-2  | Rapini site-1  | Rapini site-2

0  | 15  | 30  | 45  

site-1  | site-2
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.
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
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.
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

- Wild bees
- Alternative managed bees
- Honey bees
- Integrated Crop Pollination
  - Scientific pollination sampling
  - Economic assessment
  - Grower integration and outreach
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

2014-16 Function impact on bees and pollination
- Examine impact of mix honey bee use, managed blue orchard bee performance

ABC funded
Testing wildflower plantings in different locations within orchard

12 ft wide x 240 ft long

Orchard block

A

B

C

6 ft x 640 ft

9 ft x 6 ft

Nonpareil

Beeway
Mix Compositions

- **Almond wildflower mix**
  - Great valley phacelia
  - California blue bell
  - Five spot
  - Baby blue eyes
  - Chinese houses
  - California poppy

- **Mustard Mix**
  - Rapini mustard
  - Braco White Mustard
  - Nemfix Mustard
  - Radish

- **Clover Mix**
  - Crimson Clover
  - Hykon Rose Clover
  - Nitro Persian Clover
  - Frontier Balansa Clover
  - Alyssum

**Border plantings only**
Establishment and Flowering Success

Floral area cm²/m²

<table>
<thead>
<tr>
<th>Planting location</th>
<th>Floral area cm²/m²</th>
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<tbody>
<tr>
<td>BORDER</td>
<td>308.5</td>
</tr>
<tr>
<td>IN ROW</td>
<td>5.3</td>
</tr>
<tr>
<td>END ROW</td>
<td>2.3</td>
</tr>
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</table>

Almond wildflower mix
Bee Visitation to Flower Mixes

Visitation rate

Date

24-FEB
10-MAR
24-MAR
7-APR
21-APR

ALMOND BLOOM

Honey bee

Wild bees

Visitation rate

0
0.1
0.2
0.3
0.4
0.5
0.6

Control
Clover
Mustard
Wildflowers
Plugs

ALMOND BLOOM
Visitation to Almonds (potential competition of flower strips)

Visitation
Prop flower visited per 2 min

Mid bloom

Late bloom

Distance into orchard (meters ~yards)

Honey bees only

Without wildflower strip
Adjacent wildflower strip
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
Varroa Resistance Monitoring

Dennis vanEngelsdorp
University of Maryland
Your Newest All Natural Varroat Mite Treatment

sales@bcwagric.co.uk • 01630 655 722

MAQS®
Beehive Strip
healthy bees. healthy planet.
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) Sucrocide, and Oxalic Acid.

Some Significant Differences

[Graph showing deceased colonies by product]
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

- Nutrition
- Weather Patterns
- Queen Failure
- Genetic Weakness
- Beekeeping Practices
- Diseases
- Pesticides
- Parasites

https://www.bayercropscience.us/our-commitment/bee-health/bee-health-stressors
Honey Bee Health: Pesticide Risk Characterization

Exposure
Fate, Persistence, & Application

Toxicity
Laboratory vs. Field Testing

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
(Williams and Anderson 2013)

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®, 10.0% ai)

Coumaphos
(CheckMite+, 10.3% ai)

Amitraz
(Apivar®, 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- Uptake (Boina and Bloomquist 2009)

Voltage-Gated Cl- Channel Blocker (Jenson et al. 2013)
Natural Stilbenoid Isolate in *Photorhabdus* Bacteria of *Heterorhabditis* Nematodes

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Voltage-Gated Cl- Channel Blocker (Jenson et al. 2013)

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**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 γ-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- 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
Pest Management Challenge: Alternative In-Hive Acaricides

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

Inhibits Growth, Decreases Survival, and Reduces Cl- Uptake (Boina and Bloomquist 2009)

Voltage-Gated Cl- Channel Blocker (Jenson et al. 2013)
Field Efficacy Testing: Standard vs. Alternative Acaricides

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

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
Field Efficacy Testing: Standard vs. Alternative Acaricides

*tau*-Fluvalinate (Apistan®, 10.0% ai)
37% - 45% Efficacy (6 hr, n = 12)

Coumaphos (CheckMite+™, 10.3% ai)
26% - 51% Efficacy (6 hr, n = 12)

Amitraz (Apivar®, 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-methoxystyrlyl)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
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