Research Update: Pollination Speed Talks

December 8, 2015

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Speakers

Bob Curtis, Almond Board (Moderator)

Steve Sheppard, Washington State University

Jody Johnson, Cullaborate

Neal Williams, University of California, Davis

Ellen Topitzhofer, Oregon State University

Carolyn Breece, Oregon State University

Quinn McFrederick, University of California, Riverside

Fabiana Ahumada, Ag Science Consulting

Troy Anderson, Virginia Tech

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Bob Curtis, Almond Board

Steve Sheppard,
Washington State University

Honey Bee Stock Improvement

S. Sheppard, S. Cobey, B. Hopkins
Department of Entomology
Washington State University
Pullman WA

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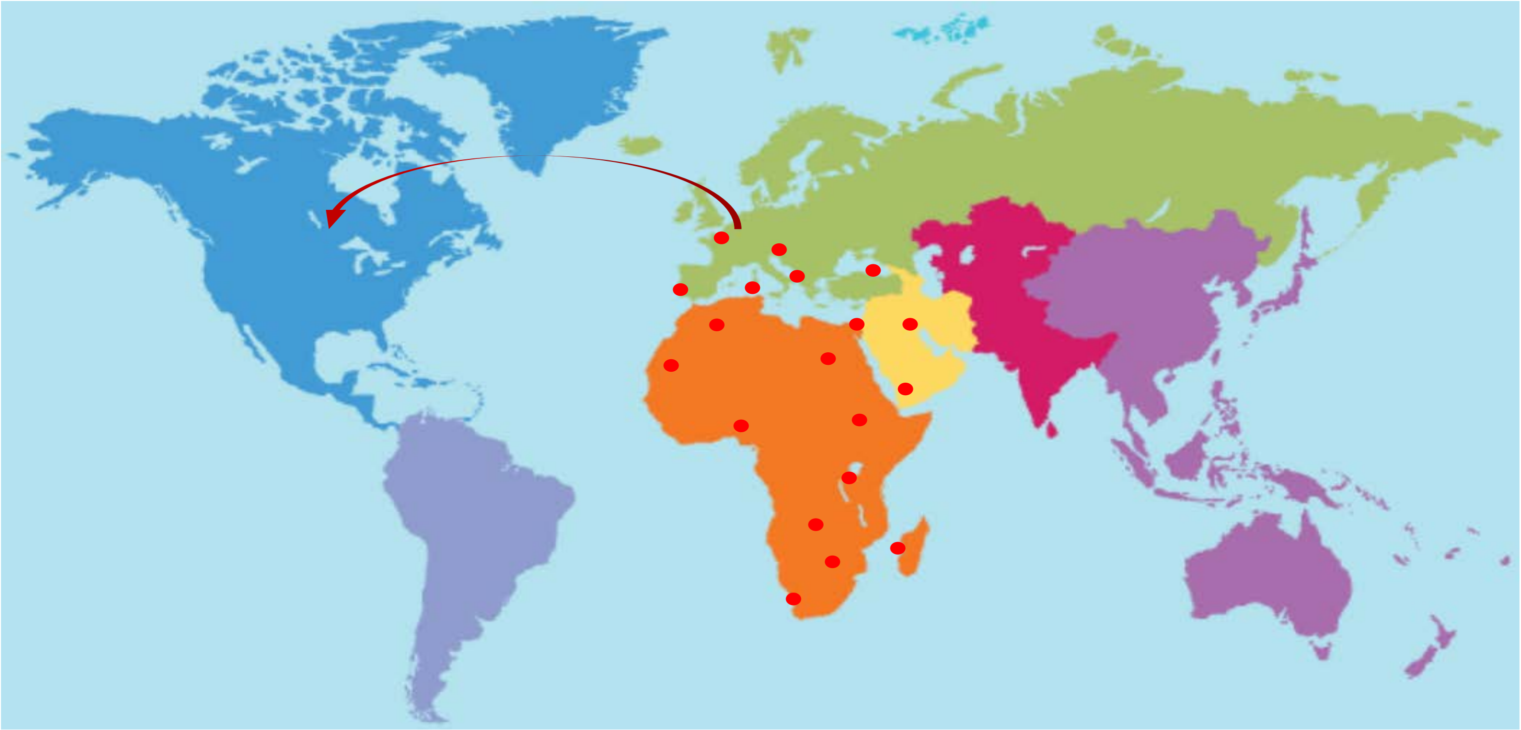
Almonds and Honey Bees – Both have overseas origins !

A subset of more than 24 Old World honey bee subspecies form the basis for all current US populations

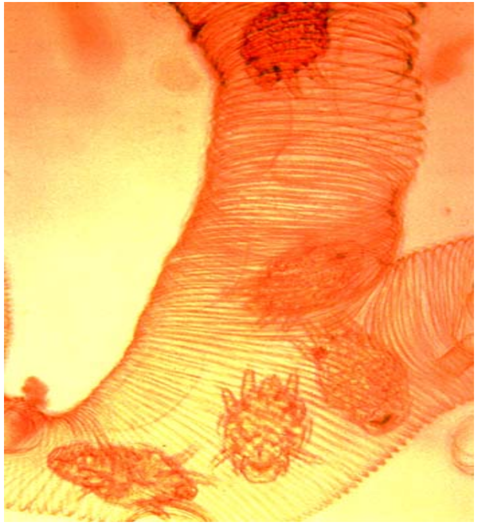


Only three introduced subspecies were maintained by US beekeepers

Subsp.	Origin	Arrival
mellifera	Europe	1600's
ligustica	Europe	1859
lamarckii	Africa	1866
carnica	Europe	1877
cypria	Middle East	1880
syriaca	Middle East	1880
caucasica	Europe	1880-1882
intermissa	Africa	1891
scutellata	Africa	1990

1922 Honey Bee Act

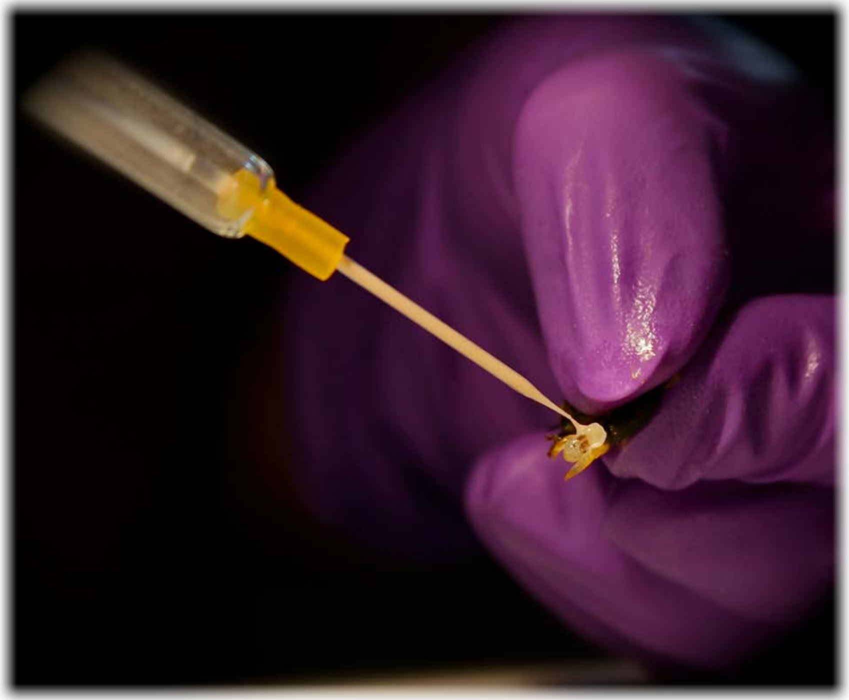
Restricted further importation of honey bees into the U.S. in an attempt to keep out tracheal mites



Honey bee breeding and stock improvement program - progress

- Importation of additional genetic diversity – Old World source populations
- Cryogenetic methods for honey bee semen
- Establishment of a honey bee germplasm repository
- Incorporation and distribution of novel genetics to honey bee queen producers
- Restablishment of Caucasian honey bees
- Introduction of a new subspecies adapted to cold weather in 2015 – *A. m pomonella*

Semen collection, cryopreservation, instrumental insemination




2015 – Tien Shan Mountains, Kazahkstan
A. m. pomonella

Questions?

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Jody Johnson, Cullaborate

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The Effect of Application Time on Fungicide Exposure to Honey bees in Almonds

Pettis, J., Bluher, S., Johnson,J., Wardell, G.

Dec. 8, 2015

Almond Board of California

Why study fungicides in honey bees?

Synergistic relationships of insecticides with fungicides (Johnson et al. 2013)

Fungicide loads in bee-collected pollen correlate with higher loads of *Nosema* (Pettis et al. 2013)

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Iprodione (a carboximide) inhibits DNA and RNA synthesis, cell division and cell metabolism.

Rovral 4F was applied by air blast ground rig at a uniform rate.

Two applications:

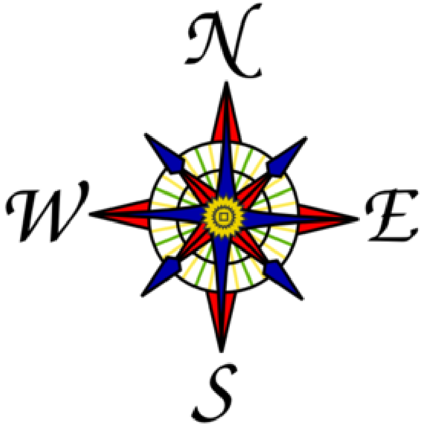
First: Site 1 at 6pm on Day 1

Second: Site 2 at 11am on Day 3.

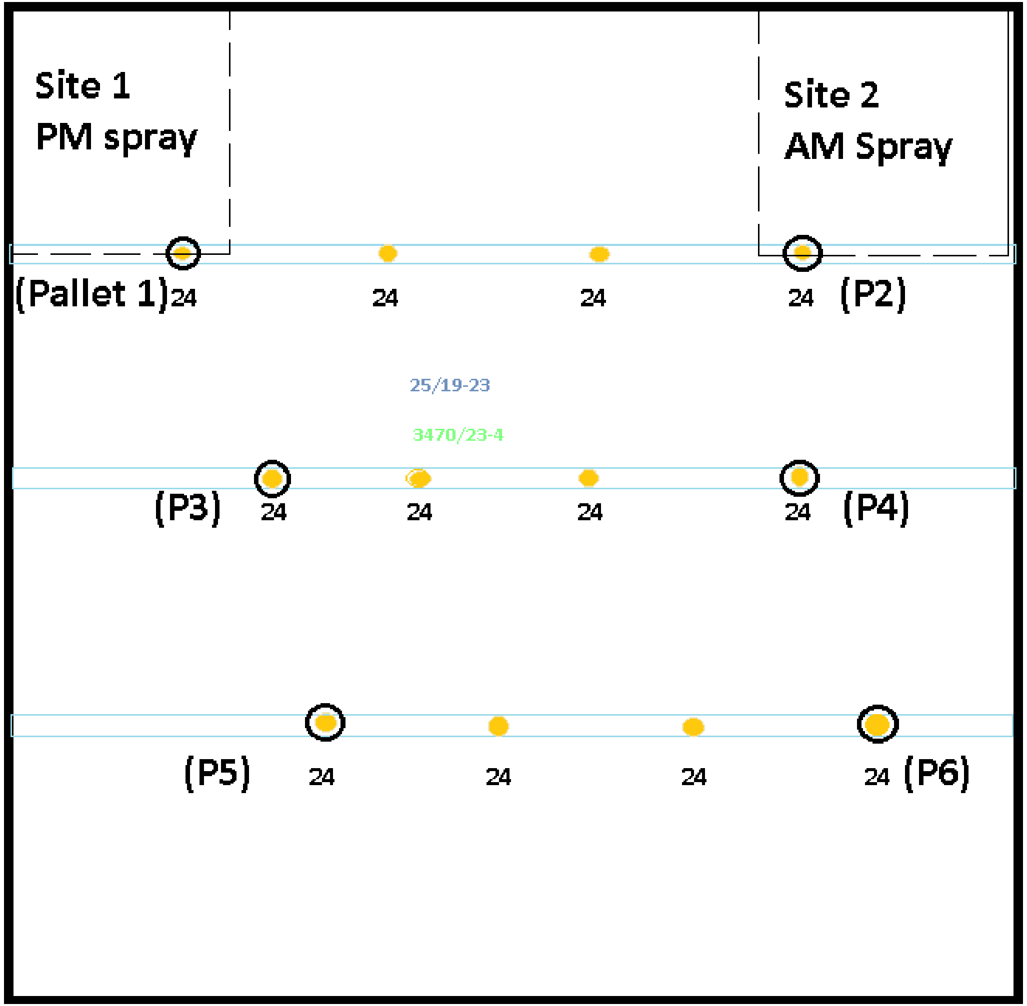
Objective

To determine if spraying fungicide at different times of day (AM vs. PM) leads to differences in the exposure levels to foraging honey bees and bee-collected pollen

Orchard map



N
W
E
S



Site 1
PM spray
Site 2
AM Spray
(Pallet 1) 24
24
24
24 (P2)
25/19-23
3470/23-4
(P3) 24
24
24
24 (P4)
(P5) 24
24
24
24 (P6)

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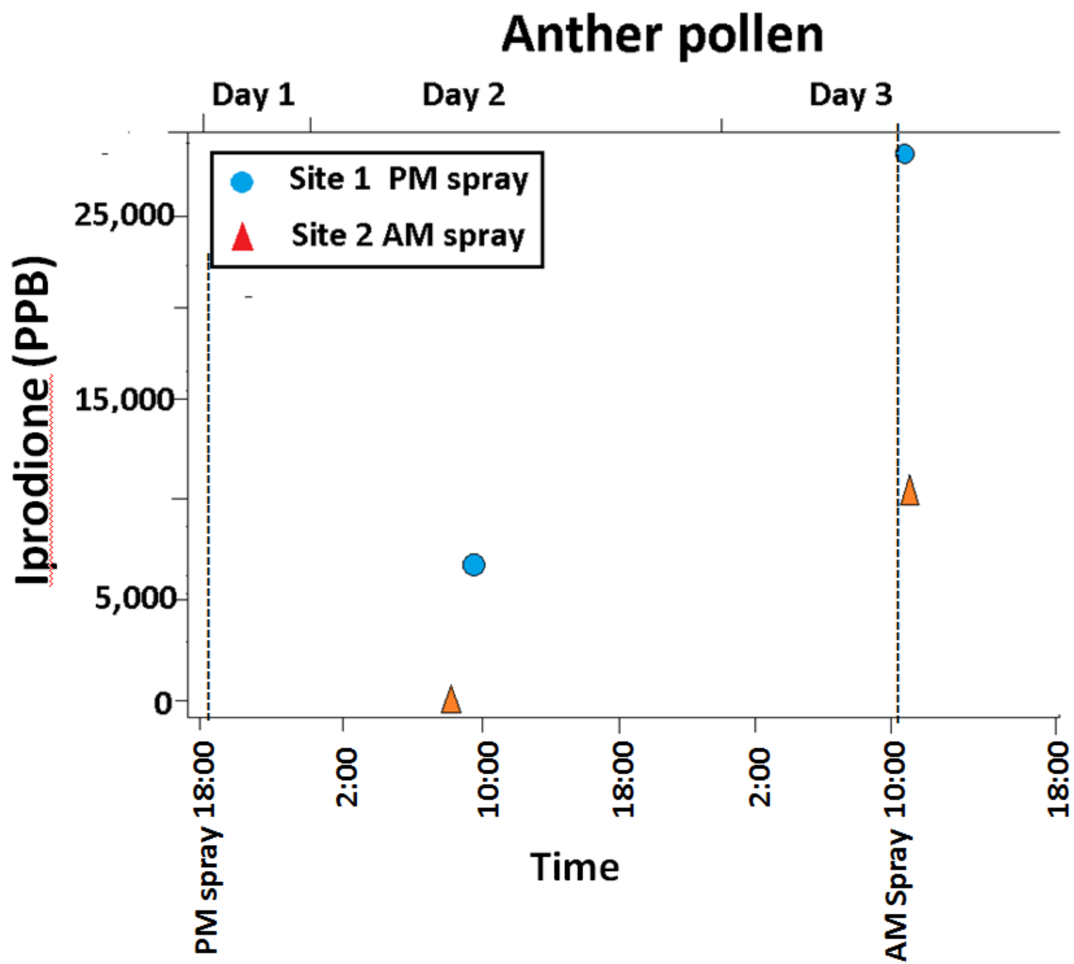
Foraging Counts

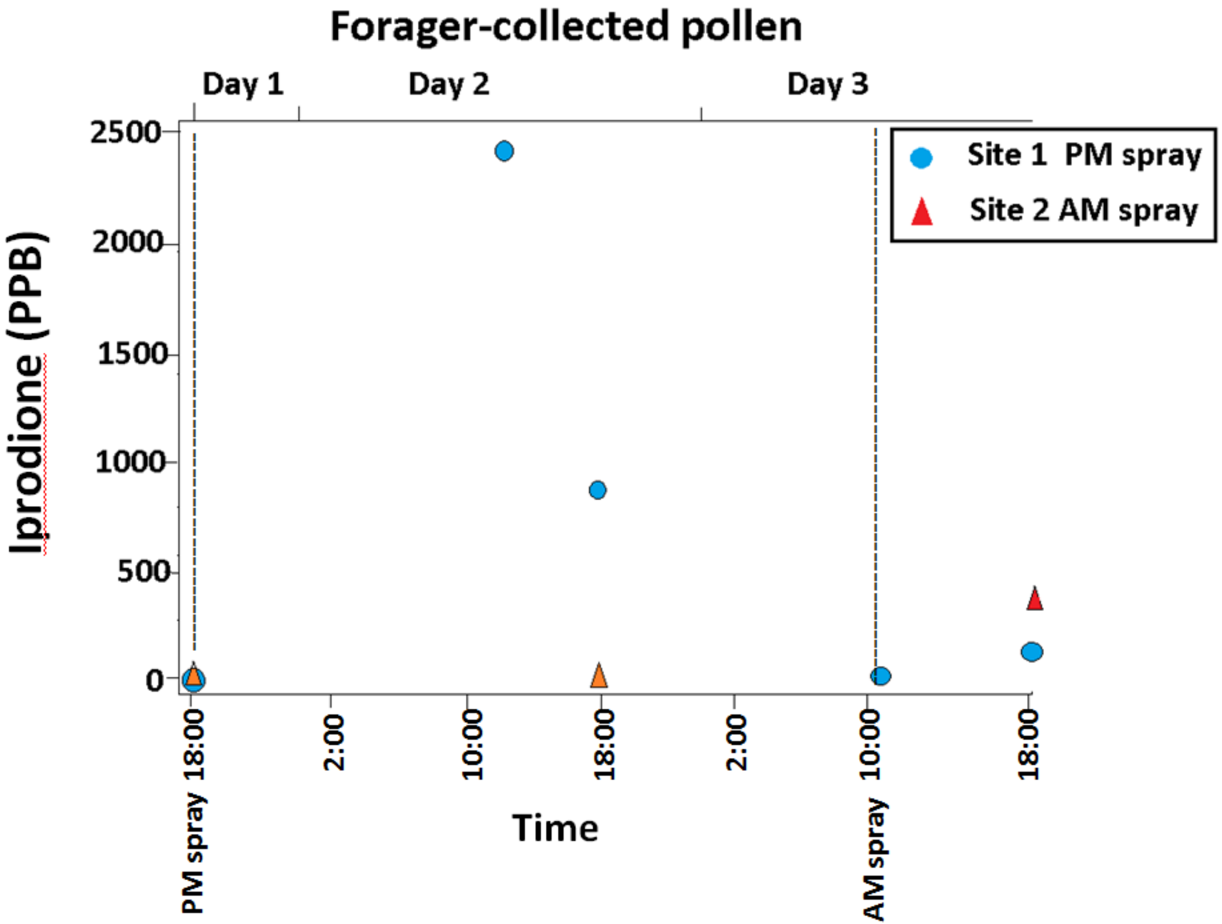
	Day 1	Day 2	Day 3
Pollen Foragers	39	27	13
Nectar Foragers	114	105	219
Foragers in Trees	6	3.6	2.8

Table 1: Mean foraging counts across the three days of study. Pollen and nectar foragers counted returning to hive entrance during three-minute interval, differentiated by presence/absence of corbicular load.

Iprodione levels (ppb) were monitored in anther pollen after spray events.


Anther pollen
Day 1
Day 2
Day 3
Site 1 PM spray
Site 2 AM spray
Iprodione (PPB)
25,000
15,000
5,000
0
PM spray 18:00
2:00
10:00
18:00
2:00
AM Spray 10:00
18:00
Time

Iprodione levels (ppb) were monitored in forager-collected pollen for the three day study



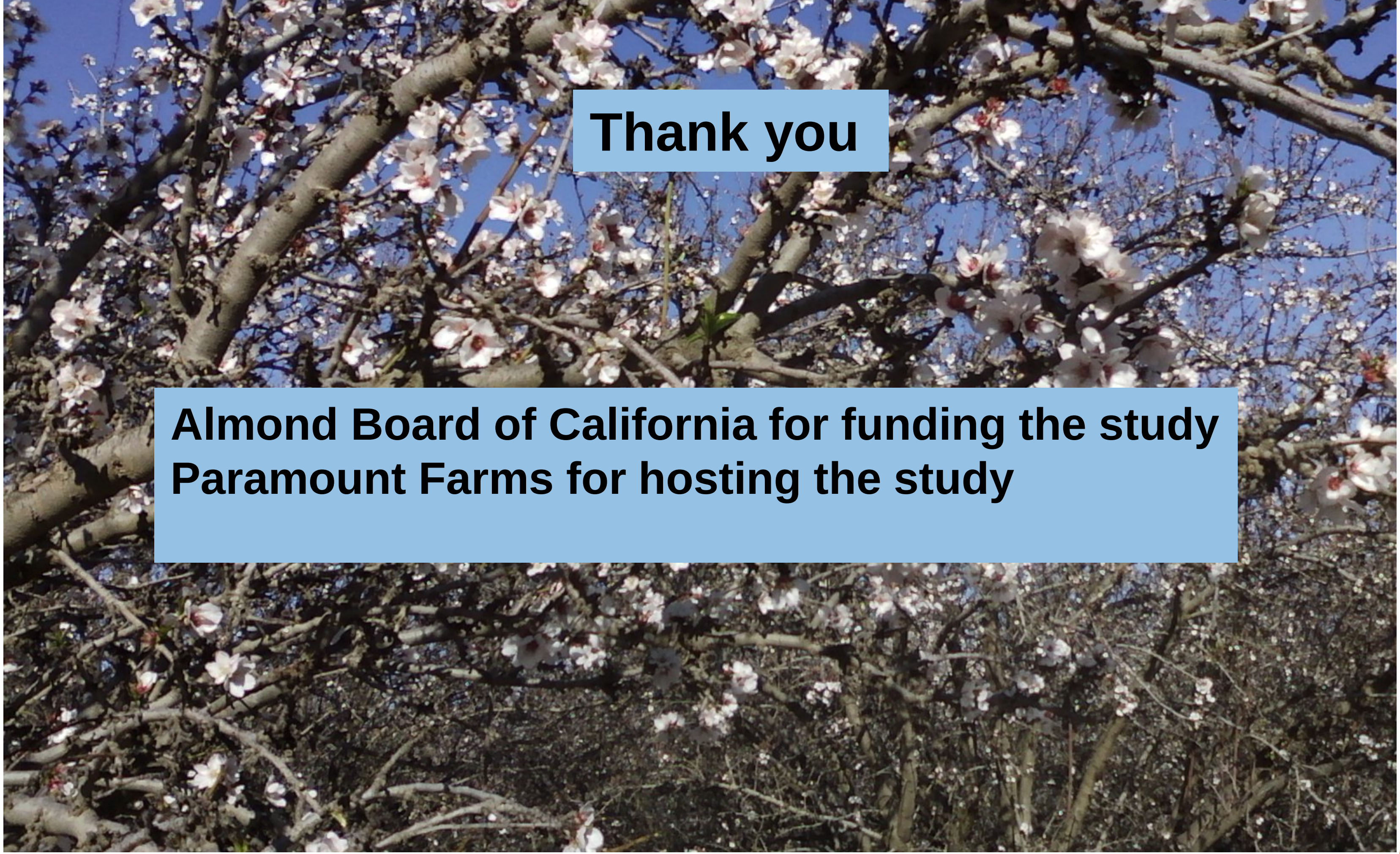
Forager-collected pollen
Day 1
Day 2
Day 3
Iprodione (PPB)
2500
2000
1500
1000
500
0
Site 1 PM spray
Site 2 AM spray
18:00
2:00
10:00
18:00
2:00
10:00
18:00
PM spray
AM spray
Time

Conclusions

- Anther pollen contained higher loads of iprodione following AM spray →Greater potential for exposure during foraging hours following AM spray vs PM spray. High iprodione in PM site area after AM spray may be due to drift.

- Actual exposure to iprodione in hives was lower following AM spray vs PM spray→decreased foraging activity or reduction of visitation to almonds due to diminishing availability of bloom.

- This study occurred during last week of bloom. Study should be undertaken during peak or consistent bloom.



Thank you

Almond Board of California for funding the study
Paramount Farms for hosting the study

Neal Williams,
University of California, Davis

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Fungicide Residual Effects on Fertilization through Stigma-Receptivity, Pollen Germination, and Tube Growth

Neal M. Williams
University of California, Davis

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PROJECT ICP

Project Apis m.

Fungicide timing- defining the issue

- Cool moist weather
- Ideal conditions for fungal pathogens
- Infection through blossoms

- Fungicides applications following rains are an integral part of best management for almond

- Optimal timing for spray application?
- Differences in impact of fungicides
- on POST POLLINATION aspects

Flower stages and fungicide exposure

Study Design

	Unexposed Drake **pollen**	Fungicide exposed Drake **pollen**
Unexposed Nonpareil **stigma**	*hand pollination (control)*	*hand pollination (pollen)*
Fungicide exposed Nonpareil **stigma**	*hand pollination (stigma)*	*hand pollination (pollen & stigma)*

Study Design

<u>Two New Fungicide Classes</u>

FRAC 3 - demethylation inhibitor

FRAC 7 - succinate dehydrogenase inhibitor

New 2016

FRAC 9 - methionine biosynthesis

FRAC 11 - quinone outside inhibitor

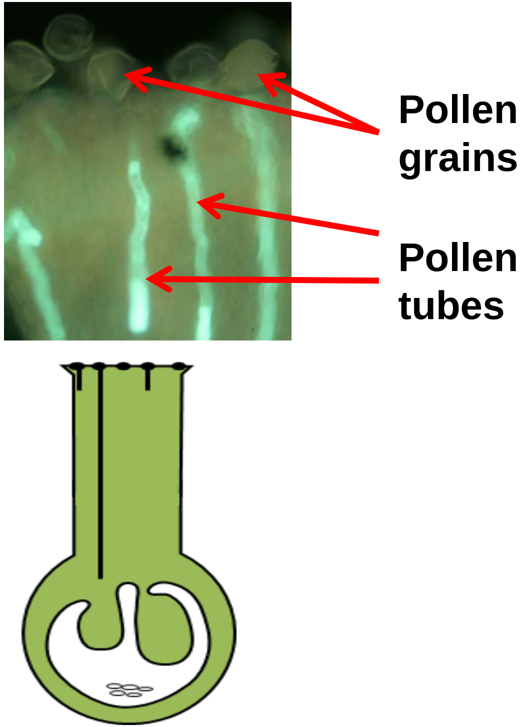
Study Methods

Assess differences in ovule fertilization of intact flowers from the different treatments

1. Timing (open flower versus in bud)

2. Before and after pollination

3. Pathways of effect: Pollen vs Stigma / style

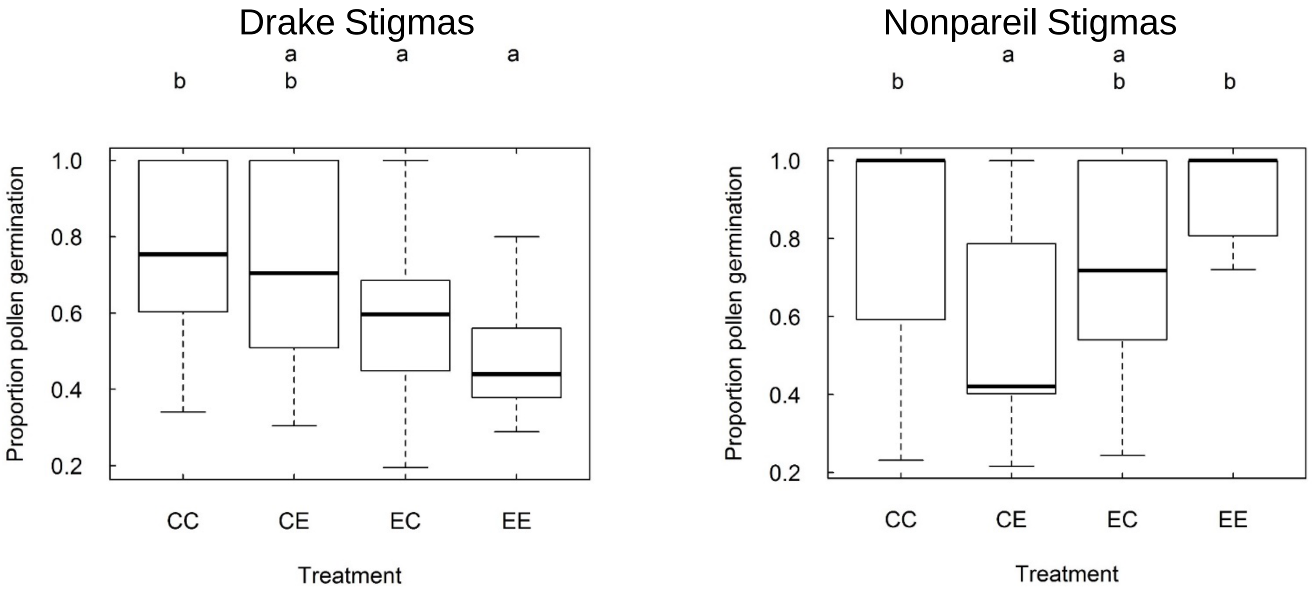


Results (2013)

Stigma variety	Chemical	Exposure	Pollen germination	Pollen tube development	No. pollen tubes ovary
Drake	FRAC 3	bud	0.210	¶0.166	0.337
Drake	FRAC 3	flower	0.321	0.878	0.506
Drake	FRAC 7	bud	**0.003**	0.655	0.982
Drake	FRAC 7	flower	0.994	¶0.969	0.952
Nonpareil	FRAC 3	bud	**0.004**	¶0.176	0.115
Nonpareil	FRAC 3	flower	0.816	0.517	0.921
Nonpareil	FRAC 7	bud	0.066	¶0.089	0.224
Nonpareil	FRAC 7	flower	0.861	0.510	0.383

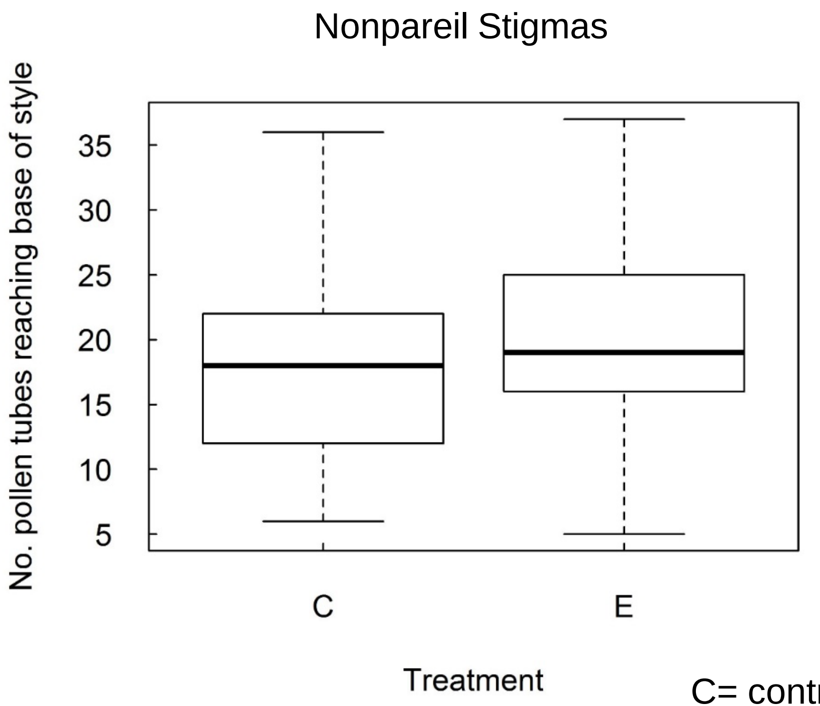
Results- Spray precedes pollination

Pollen germination



CC= control, CE= control stigma exposed pollen, EC= exposed stigma control pollen, EE= exposed stigma exposed pollen

Results- Post-pollination sprays



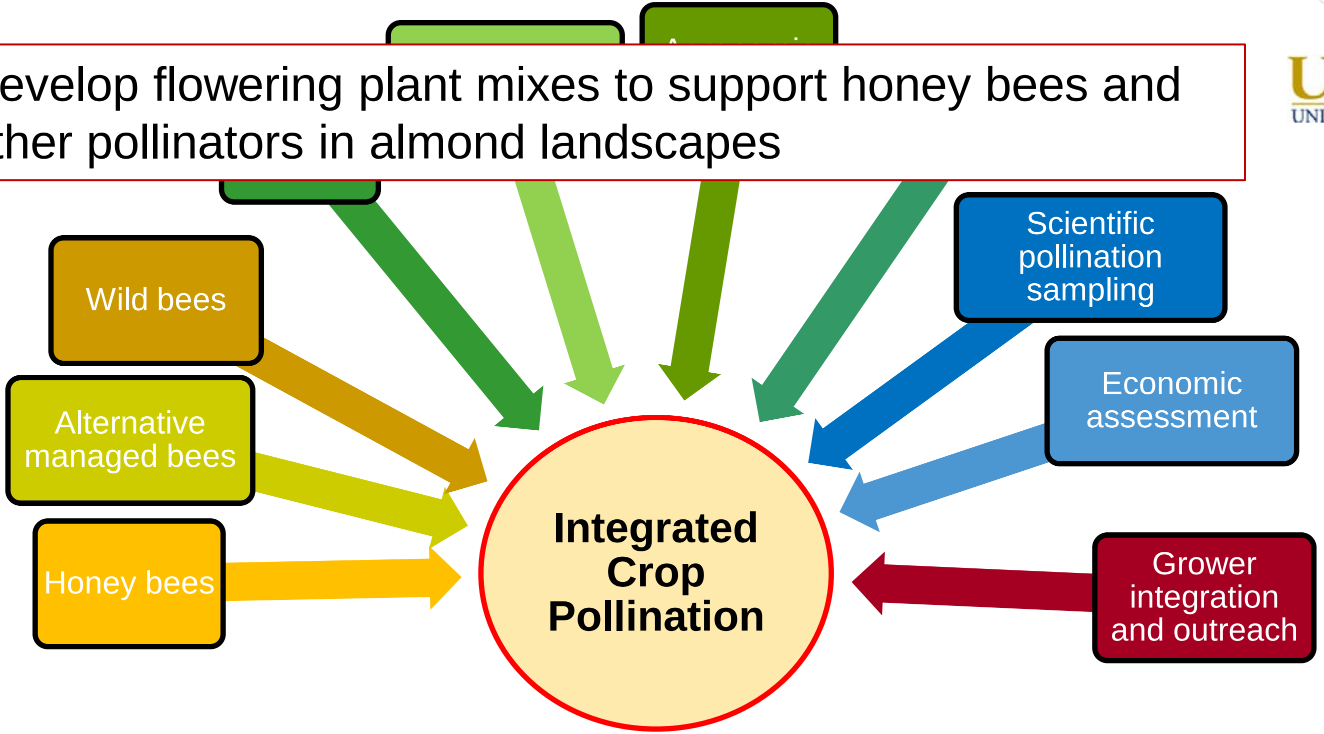
C= control , E= exposed stigma

Summary

- Impacts of FRAC 3 and FRAC 7 fungicides are modest and inconsistent
- Decrease in pollen germination, does not persist through fertilization
- Streamline method for fungicide testing that could be easily and more widely applied to new sprays
- Additional FRAC groups 9, 11 will be tested

Forage for honeybees: Integrated Crop Pollination

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



Highlights

- **Mustard and wildflower** mixes provided the **most bloom** and wildflower flowering persisted longer after almond flowering
- **Mustard** and **wildflower mix attracted** the **most honeybees**
- **Wildflower** mix, then mustard **attracted** the **most wild bees**
- Mixes **did not** attract honey bees away from the orchard flowers

Ellen Topitzhofer, Oregon State University

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Tech Transfer Teams for Commercial Beekeeping: Pacific Northwest Team

Ellen Topitzhofer

PI: Ramesh Sagili

Oregon State University

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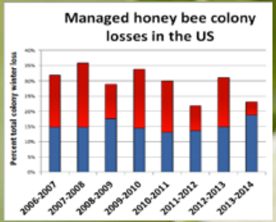
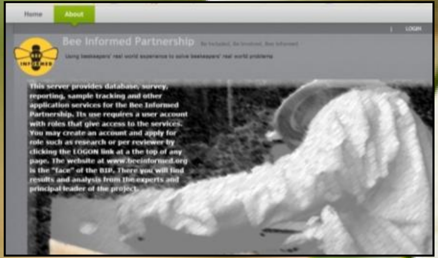
The Bee Informed Partnership

Using beekeepers' real world experience to solve beekeepers' real world problems

On-the-ground testing

Surveys





Database

USDA ARS

APHIS

National historic and
on-going diagnostic data

USDA NIFA
United States Department of Agriculture
National Institute of Food and Agriculture

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4 visitations for our commercial beekeepers

Treatment | | Treatment |
|---|---|---|---|
| | | | |

1 — Feb/ March

Treatment ↓

2 — April/ June

3 — July/ August

Treatment ↓

4 — Sept/ Oct

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Sample Types

- Varroa/Nosema: % Varroa mite infestation and Nosema spp. spore count
- Viruses: quantify levels of 7 viruses (NC State)
- Protein: head protein content (OSU)
- Queen quality: sperm viability and count (NC State)
- Disease (ABF/EFB): presence/absence (USDA-Beltsville)
- Pesticides: quantify and report as PPB (USDA-Gastonia)

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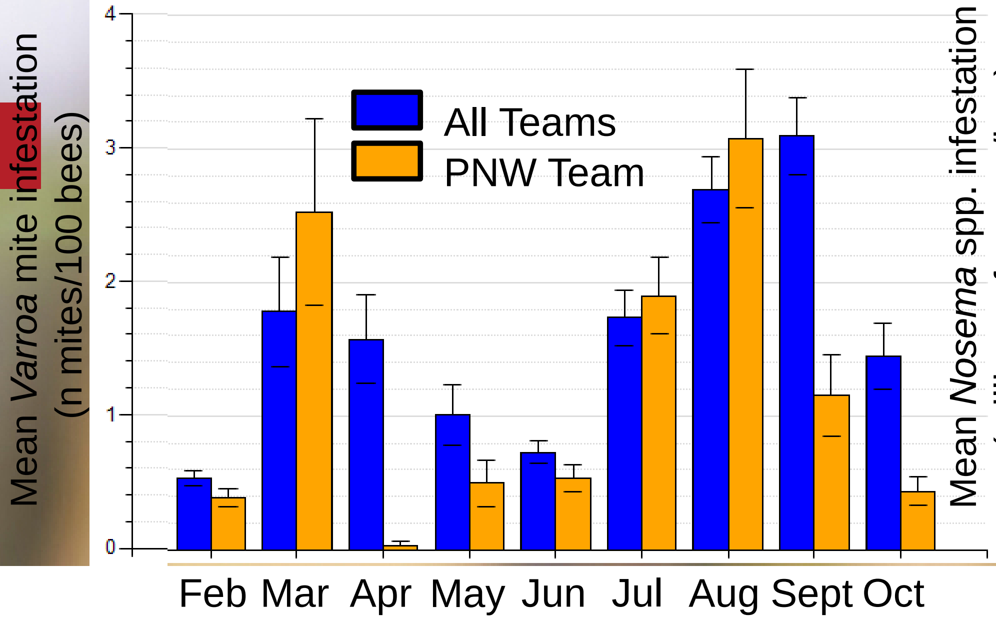
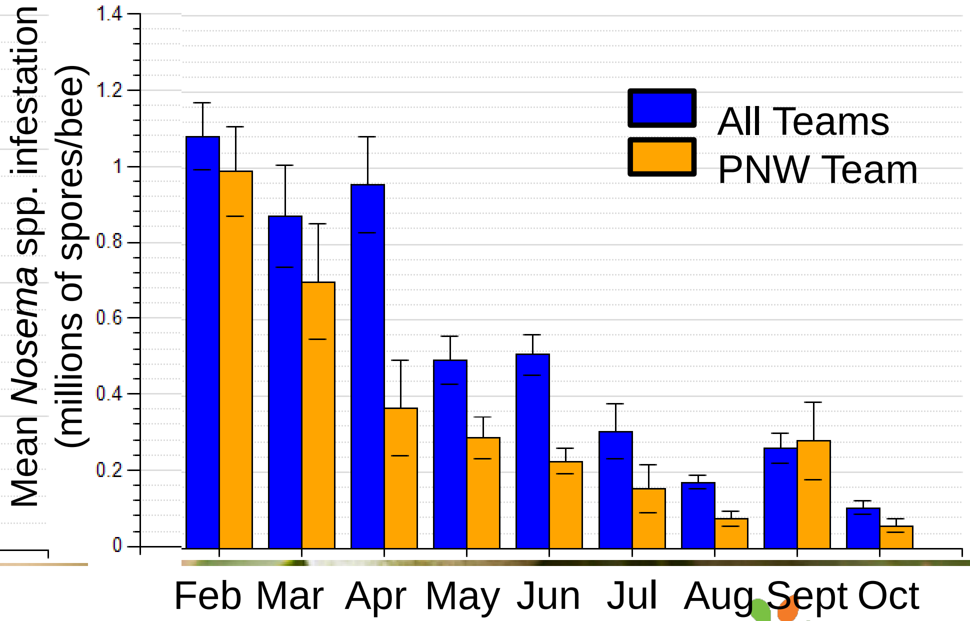
2015 Varroa sample levels



2015 Nosema levels



Error bars indicate 95% CI

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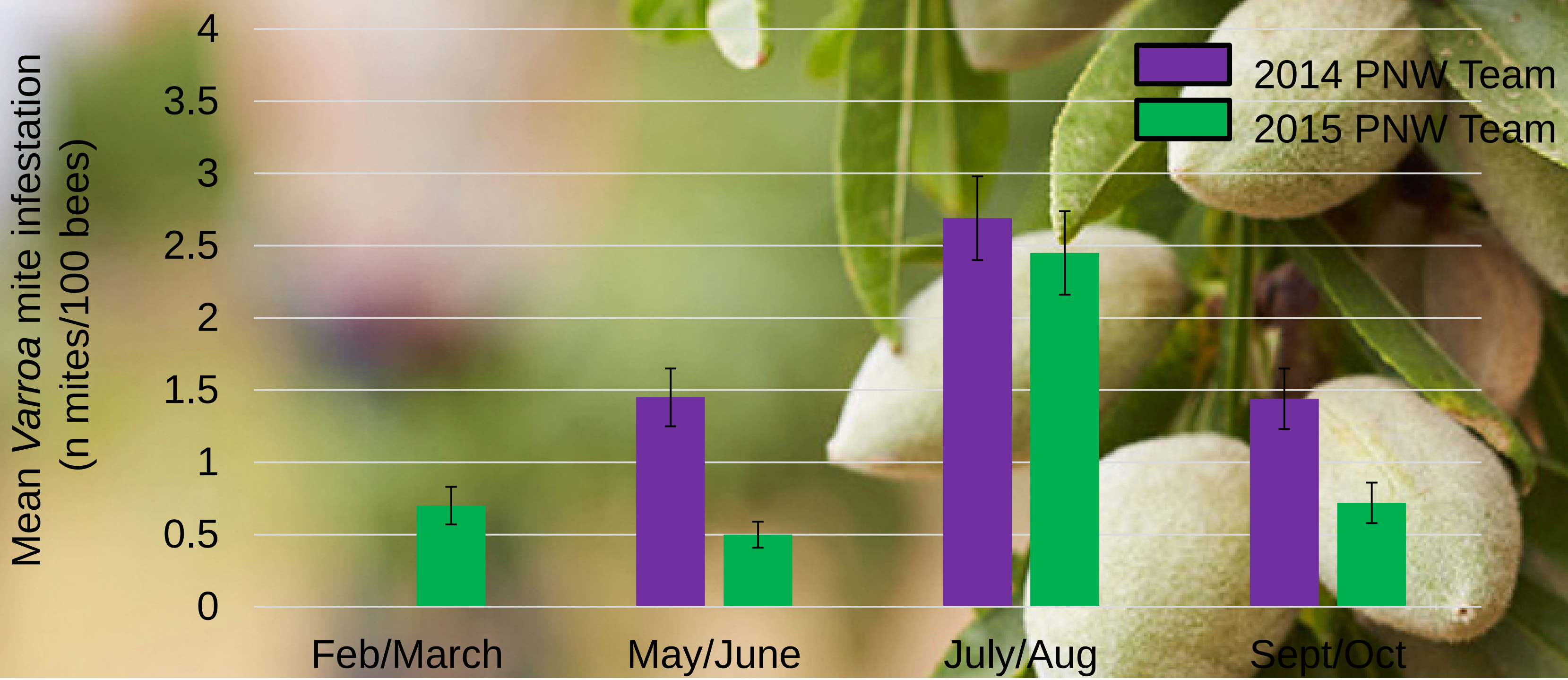
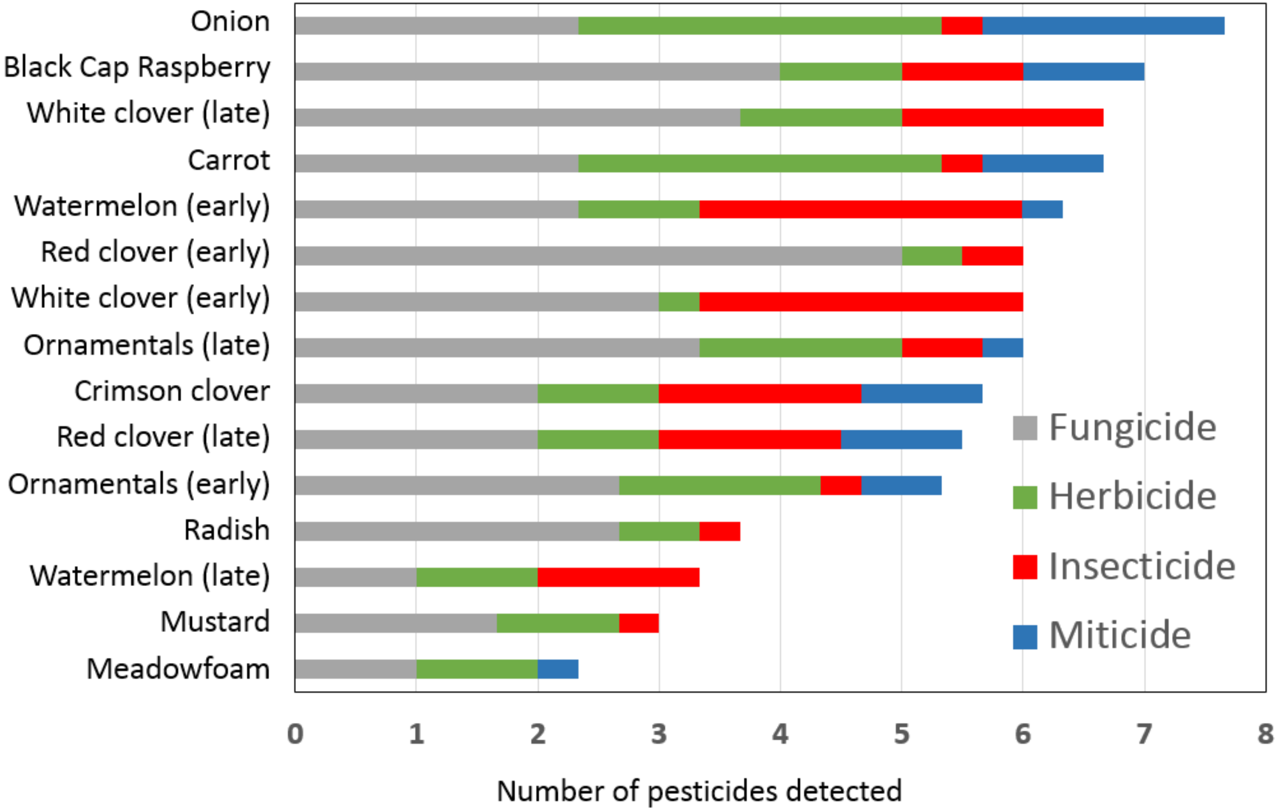
2014 vs. 2015 Varroa levels

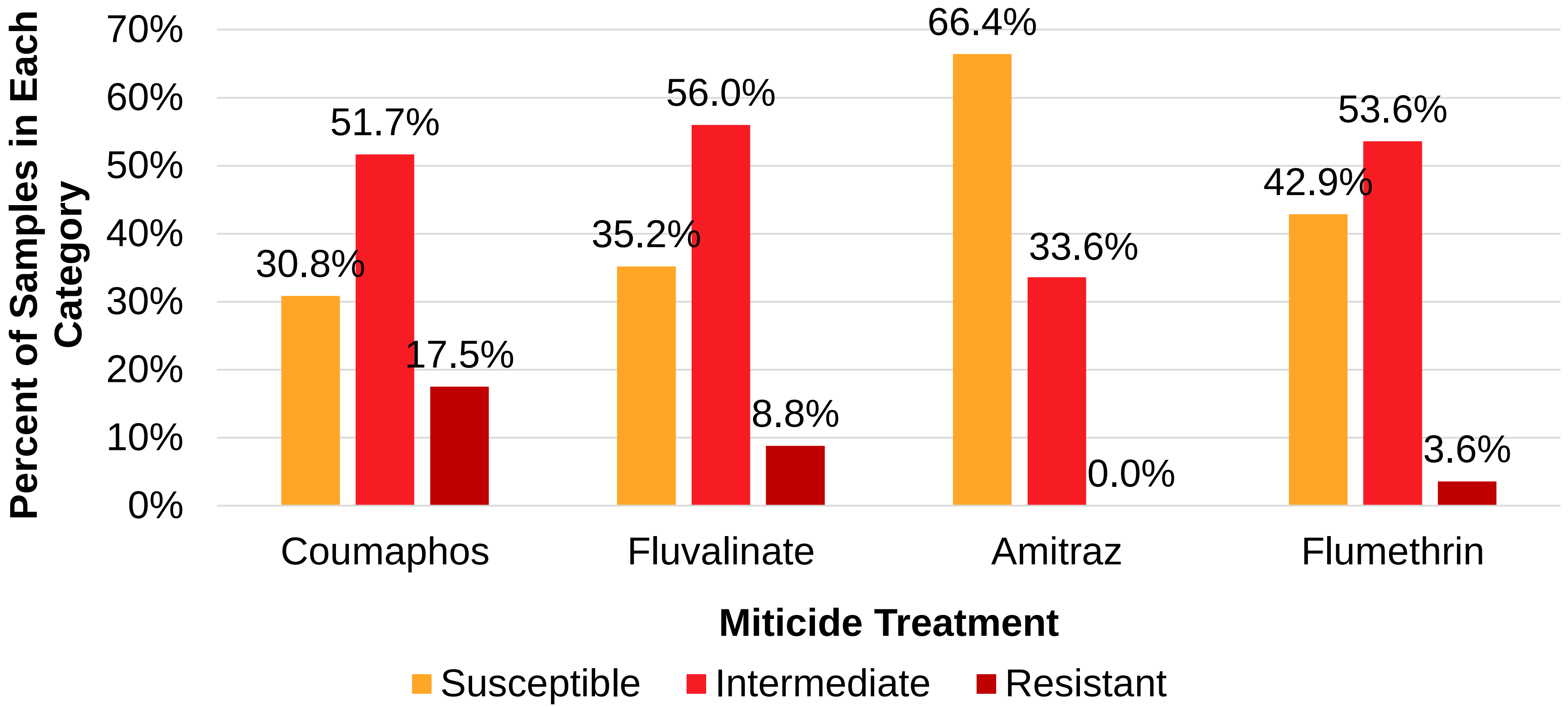


Error bars indicate 95% CI

Total pesticides detected per field



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



Thank You!

USDA NIFA

United States Department of Agriculture
National Institute of Food and Agriculture

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OSU
Oregon State
UNIVERSITY

AIA Apiary Inspectors of America

APHIS

AHPA

ABF
1943
American Beekeeping Federation

HoneyBeeNet

Project Apis m.

Bee Colony Losses
Prevention of Honey

USDA ars United States Department Of Agriculture
Agricultural Research Service

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Carolyn Breece,
Oregon State University

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Assessing the value of supplemental forage for honey bees during almond pollination

Ramesh Sagili and Carolyn Breece
Oregon State University

Planting supplemental forage for honey bees

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

Methods

Mustard plantings (Project Apis m.)

Wildflower plantings (UC Davis)

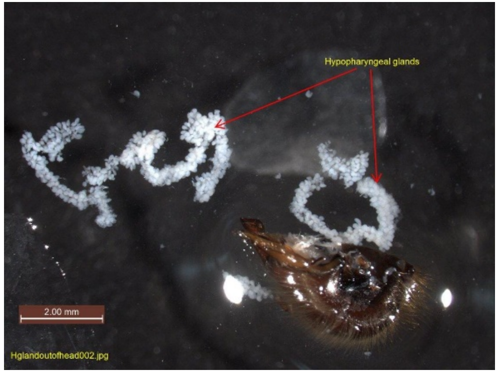
Methods

- Colony evaluations
- Pollen traps
- Honey bee samples
- Lab analysis

Results

We analyzed bee samples for

- varroa mites
- nosema
- protein content (measure of nutrition)
- immune system enzymes



Results were highly variable.

Results: Pollen identification and proportion

Wildflower	Site	Pollen	23 Feb	27 Feb	6 Mar	10 Mar
Far	MA	Almond	94	76	6	
		Wildflowe	3	9	42	
		mustard	0	7	6	
		Unknown	3	5	20	
		Weed	0	3	31	
Near	MW	Almond	72	58	0	0
		Wildflowe	7	15	8	23
		mustard	16	42	23	57
		Unknown	4	6	6	2
		Weed	1	9	66	18

Mustard			12 Feb	21 Feb	12 Mar
Far	HA	Almond	97	96	76
		mustard	0	0	14
		Unknown	3	4	86
		Weed	0	0	0
	KA	Almond	91	50	0
		mustard	1	48	91
		Unknown	8	3	12
		Weed	0	0	1
Near	KM	Almond	91	31	0
		mustard	0	66	64
		Unknown	10	8	36
		Weed	0	0	0
	PM	Almond	93	31	1
		mustard	3	73	83
		Unknown	4	6	77
		Weed	0	0	0

Tables courtesy of Kimiora Ward and Neal Williams, UC Davis

Thank you

Our collaborators:

- Dr. Neal Williams and Kimiora Ward, U.C. Davis
- Project Apis m.
- Wonderful Farms
- Beekeepers from California and Oregon
- Almond growers

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

Quinn McFrederick,
University of California, Riverside

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The influence of cover crop forage on honey bee nutrition and gut microbes, and on colony growth and activity

Quinn McFrederick[1], William Meikle[2], Mark Carroll[2]

1. UC Riverside Department of Entomology
2. USDA Carl Hayden Bee Research Center

Objectives and methods

Objectives: Determine effects of supplemental rapini forage on honey bee:

1) Nutrition, health, and queen quality

2) Brood production

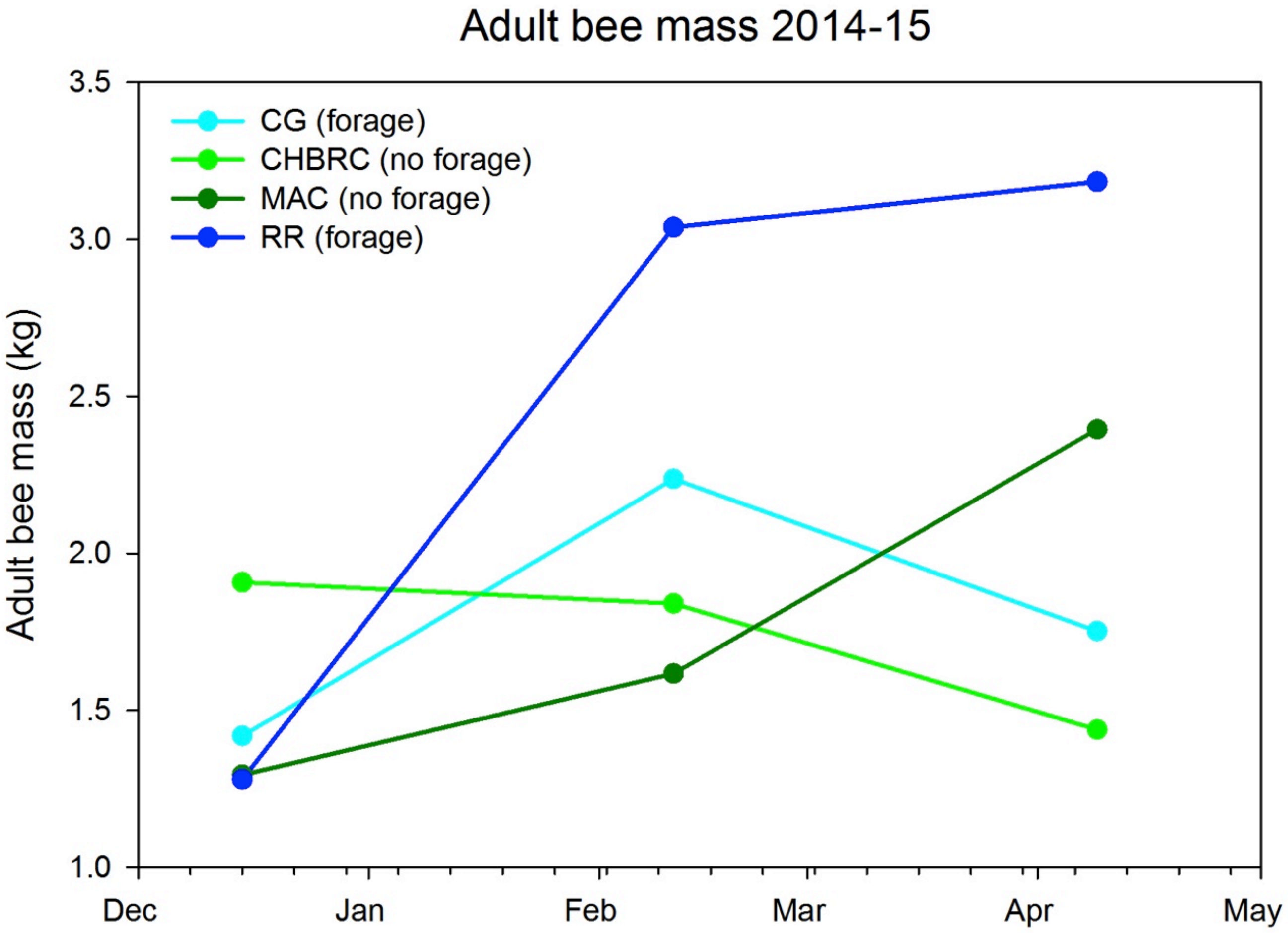
3) Gut microbiome

4) Interactions between all these factors

- Methods
  - 40 colonies
  - 2 forage and 2 non-forage plots in AZ
    - mid January
  - Moved to Almonds
    - mid February

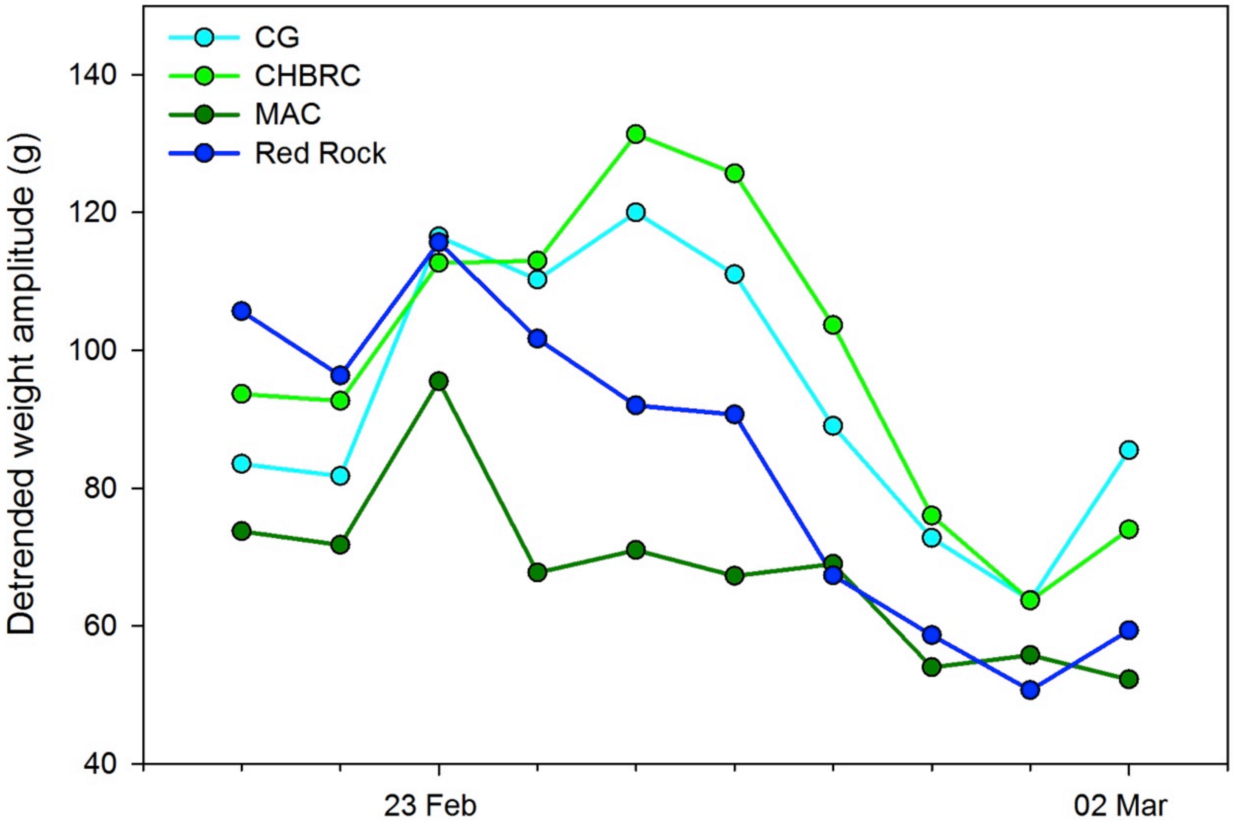
Results

Average adult bee mass



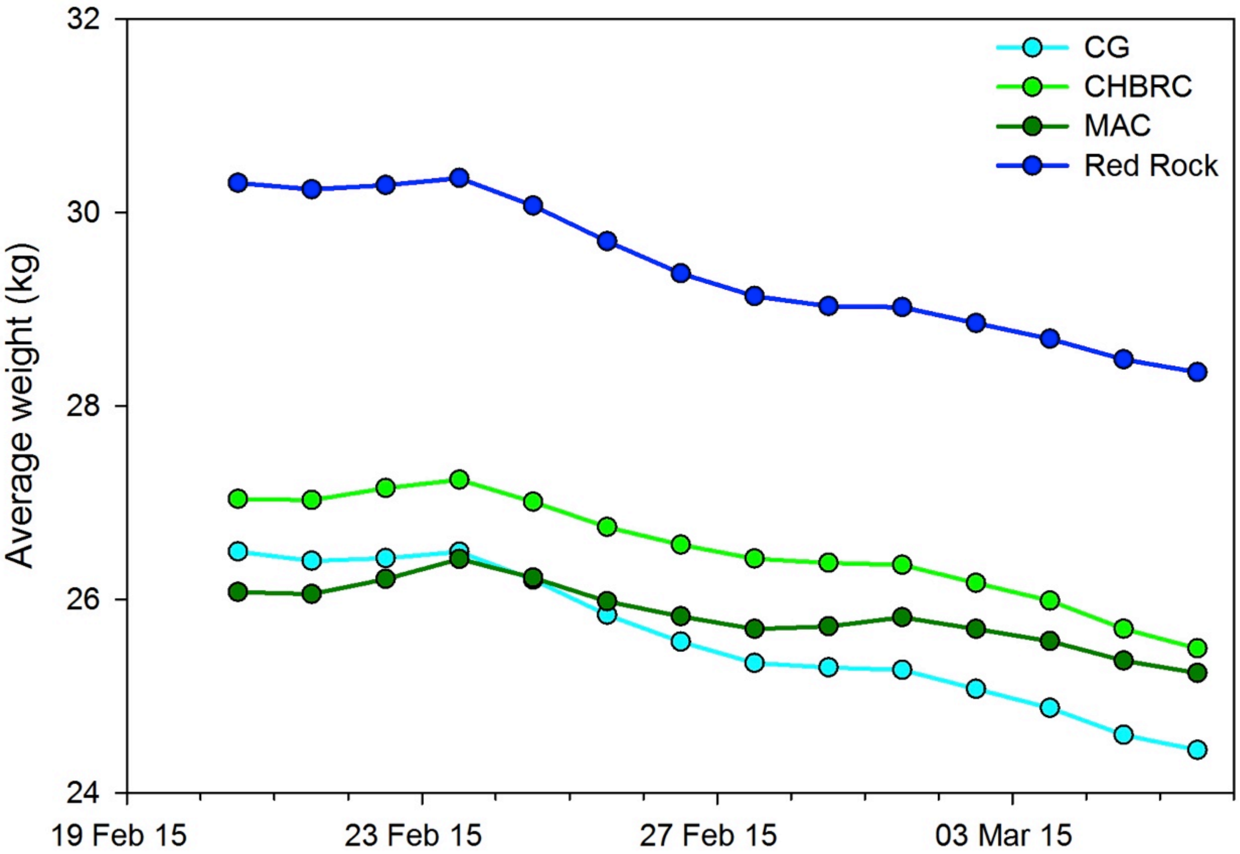
Results

Daily colony weight variation during almond pollination



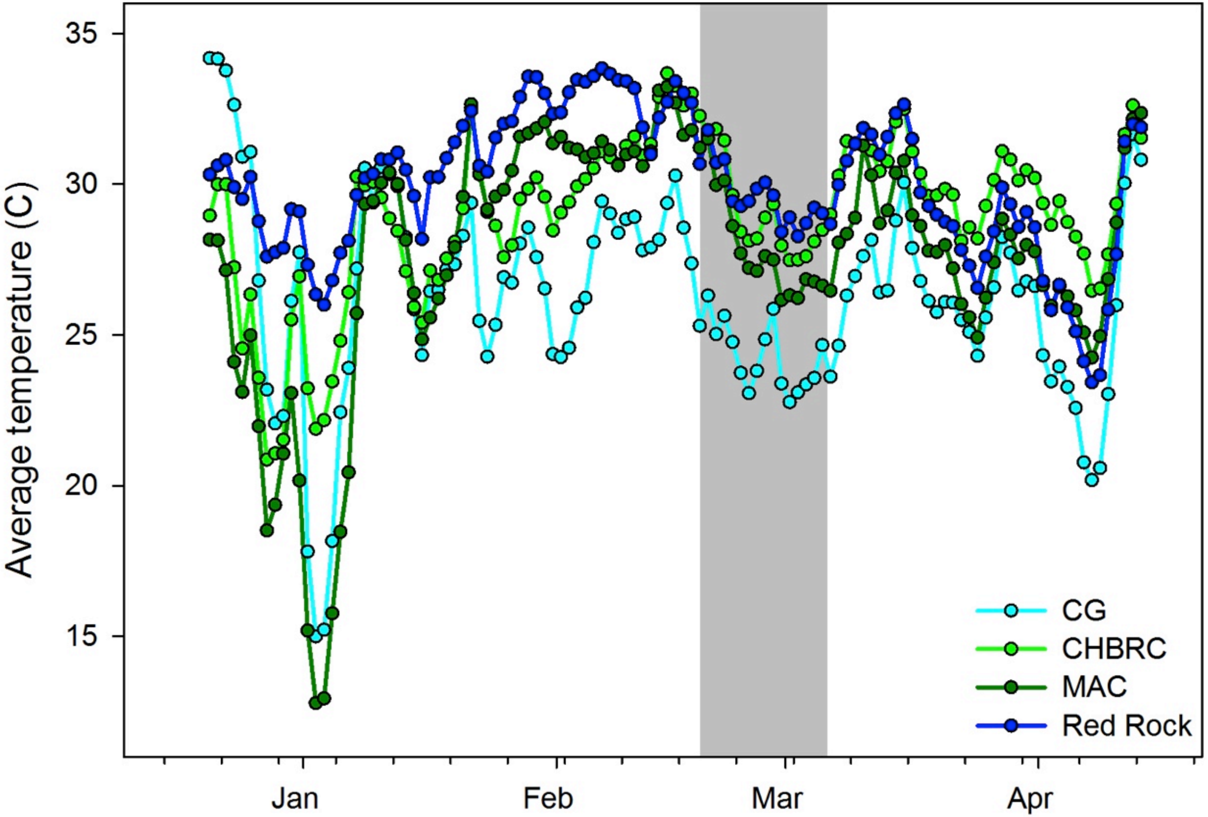
Results

Average weight during almond pollination



Results

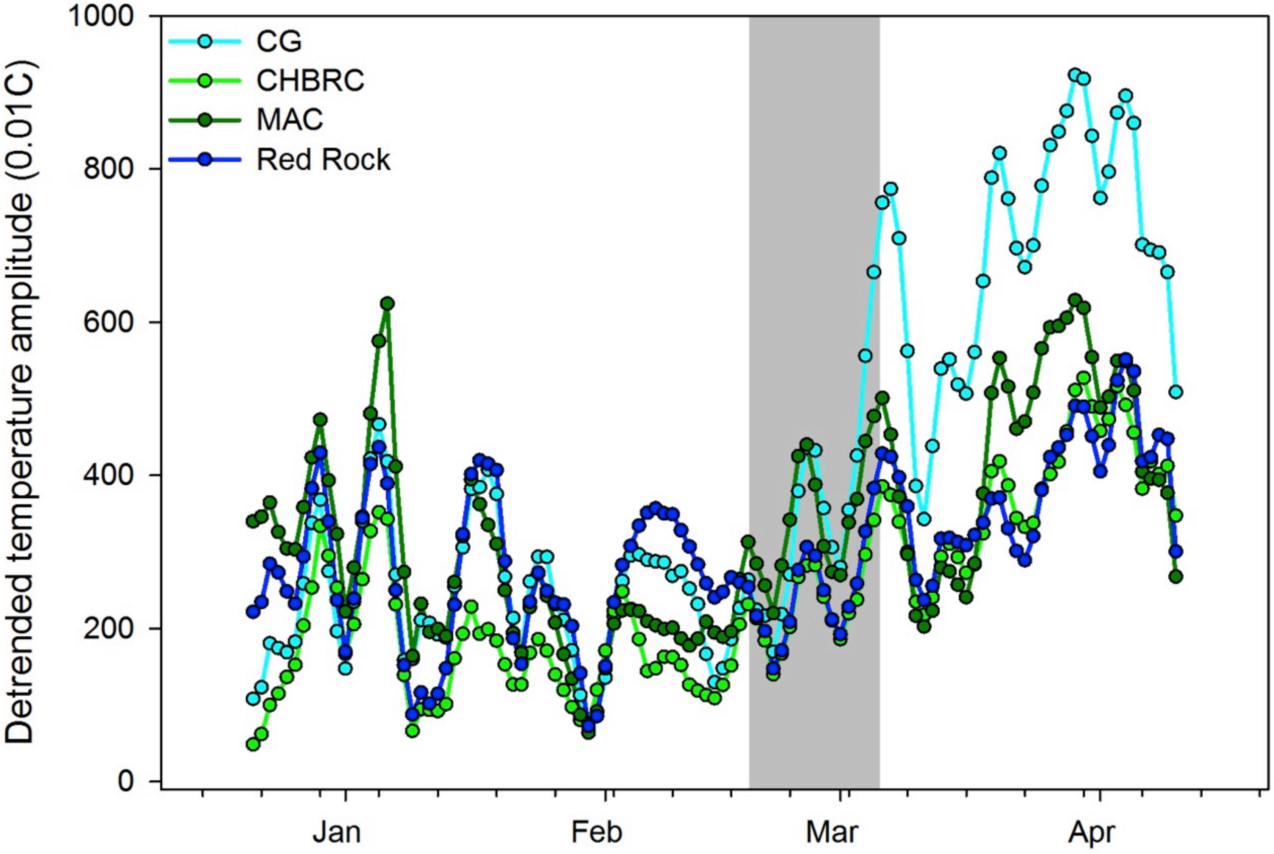
Average temperature inside the colony



Results

Average daily temperature variation

- Lower numbers indicates brood rearing



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Conclusions

- Forage treatment did not increase weight
  - Caveats
    - Non-forage sites had pollen coming into hives
    - Forage treatment not as long as planned
- Site matters
  - Red Rock colonies had more foragers and greater weight
- Nutrition and gut microbe work ongoing
  - 1,100 honey bees dissected
  - ~200 samples awaiting DNA sequencing
  - Queen quality

Thanks to Milagra Weiss, Nick Brown, Jason Rothman, and Wonderful Farms for logistical support, and to you for your support.

Fabiana Ahumada,
Ag Science Consulting

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Implementing an Integrated Pest Management for *Varroa*

Fabiana Ahumada

AgScience Consulting

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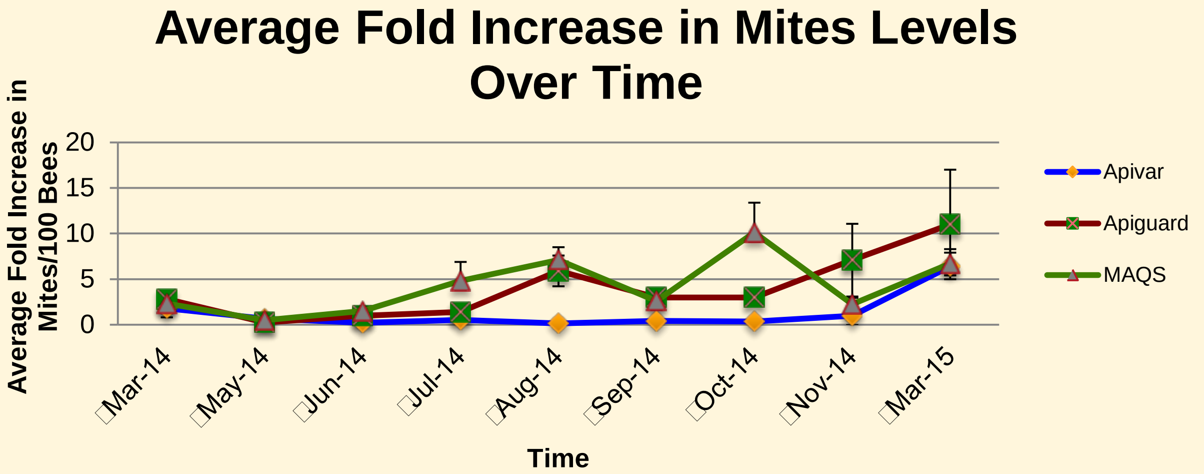
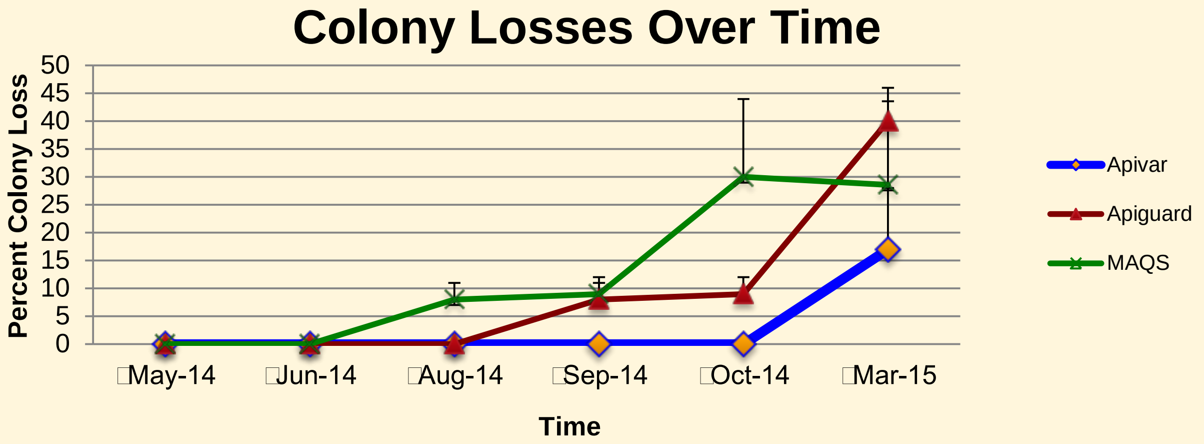
Project Overview

Implement an Integrated Pest Management program for Varroa control

- 2014-2015:
  - Determined mite treatment efficacy
  - Treatment effect on colony strength
  - Colony losses
- 2016:
  - Install and establish bee packages
  - Implement a mite treatment regime
  - Design an IPM program

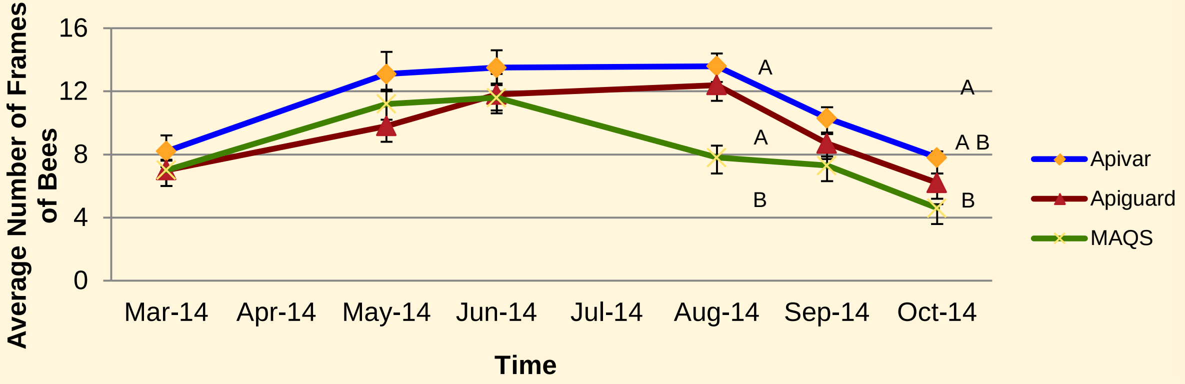


Average Fold Increase in Mites Levels Over Time
Average Fold Increase in Mites/100 Bees
20
15
10
5
0
Mar-14
May-14
Jun-14
Jul-14
Aug-14
Sep-14
Oct-14
Nov-14
Mar-15
Time
Apivar
Apiguard
MAQS

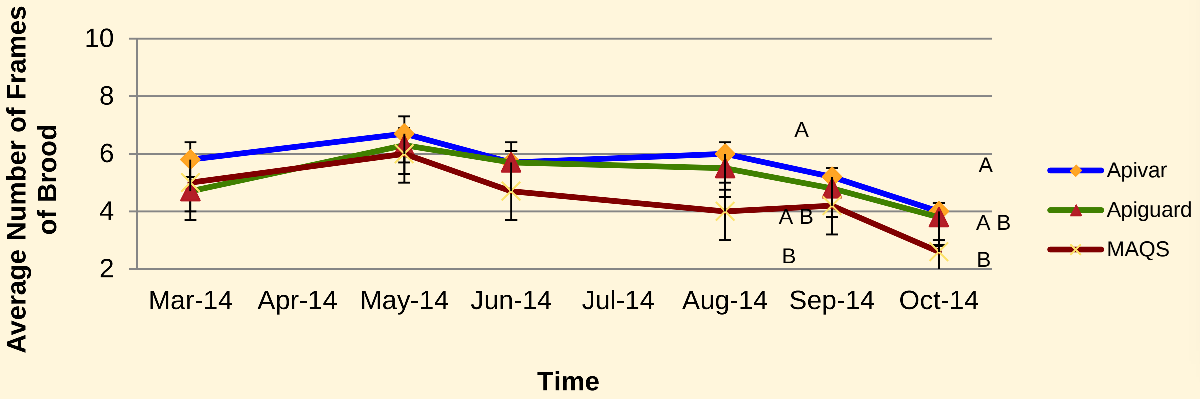


Colony Losses Over Time
Percent Colony Loss
50
45
40
35
30
25
20
15
10
5
0
May-14
Jun-14
Aug-14
Sep-14
Oct-14
Mar-15
Time
Apivar
Apiguard
MAQS

Frames of Bees Over Time



Frames of Brood Over Time



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Next Phase

- Install and establish bee packages
- Varroa levels baseline
- Apply Spring, Summer and Fall mite treatments
- Monitor mite levels and colony strength
- Design an Integrated Pest Management Program for Varroa control

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Acknowledgements

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Gene Brandi’s Apiaries

Carl Hayden Bee Research Center

Troy Anderson,
Virginia Tech

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Discovery of Stilbene Chemistries for Varroa Mites

Troy D. Anderson, PhD
Department of Entomology, Virginia Tech

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Pesticide Risk Characterization for Honey Bees

Exposure

Fate, Persistence, & Application

x

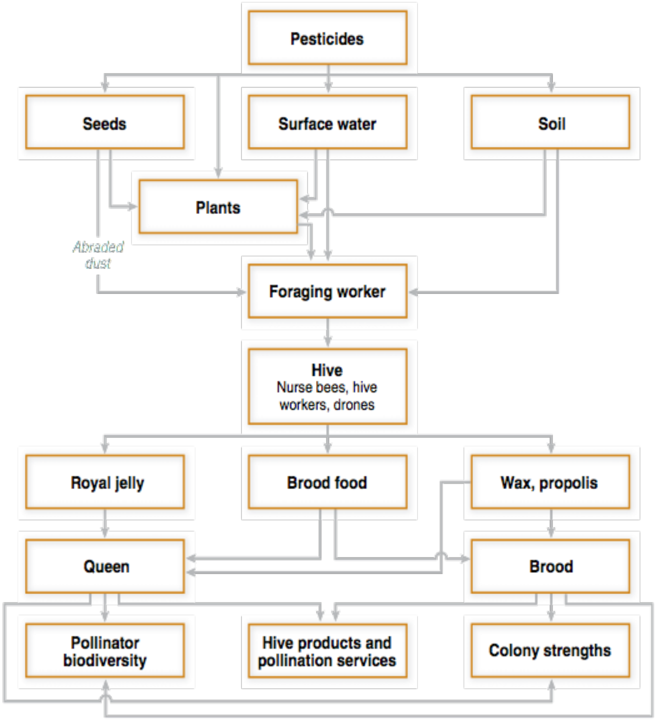
Toxicity

Laboratory vs. Field Testing

=

Risk

Predict Effects of Pesticide Use, Misuse, & Safety



Fairbrother, Anderson et al. 2014

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Honey Bee Colony Losses for Apiculture Industry

Bee Colony Losses:

~ 30% in Virginia

$1.3-1.8 Million Lost:

Pollination Fees

Honey Sales

Colony Replacement

Multiple Stressors,
Multiple Interactions

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News » Nation · Demographics · Troops at Risk · Lotteries

U.S. losing bees and beekeepers

Updated 4/9/2008 11:14 AM | Comments 8 | Recommend 8

Enlarge — By Kalim A. Bhatti, USA TODAY

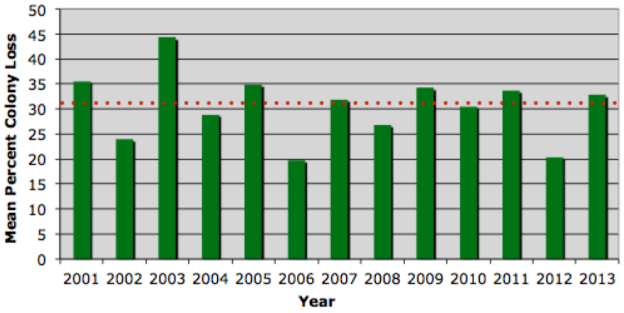
Beekeeper David Hackenberg works on his hive in Lewisburg, Pa., April 29, 2007. Hackenberg has lost nearly $400,000 from the mysterious bee deaths across the country.

By Heather Collura, Special for USA TODAY

The number of bees is on the decline across the USA, and there's also a shortage of beekeepers.

The number of commercial beekeepers is dwindling because the business of keeping bees is not as profitable as it once was, according to Jeff Pettis, research leader at the U.S. Department of Agriculture Bee Research Laboratory in Maryland.

That decline in profitability is due in large part, Pettis said, to lower honey prices — the average U.S. price per pound dropped four-tenths of a cent over the past year. Keepers also face difficulty in keeping healthy bees



Honey Bee Colony Losses for Apiculture Industry

Bee Colony Losses:

~ 30% in Virginia

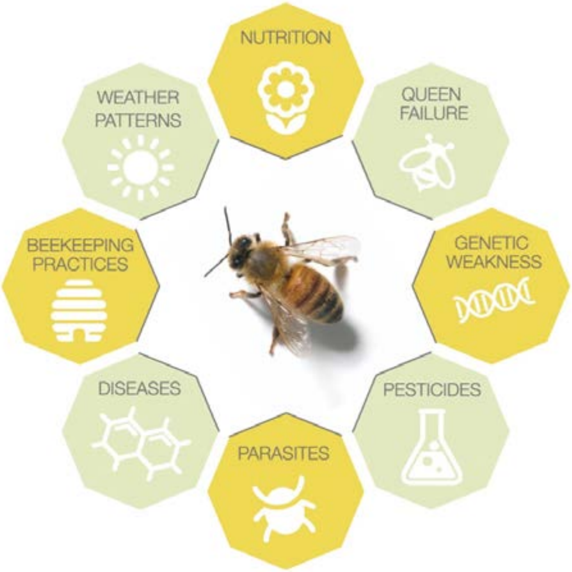
$1.3-1.8 Million Lost:

Pollination Fees

Honey Sales

Colony Replacement

Multiple Stressors,
Multiple Interactions



Credit: Bayer CropScience

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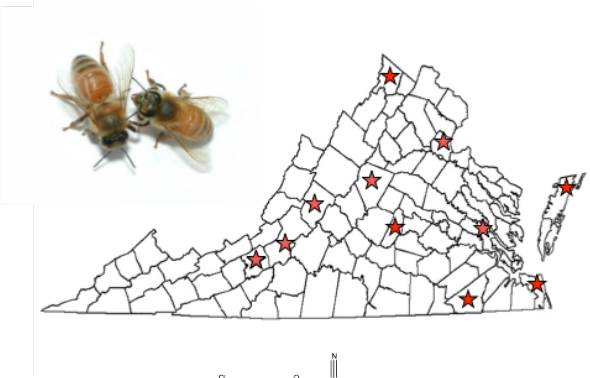
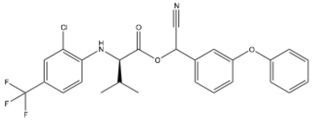
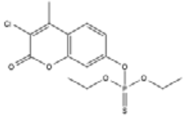
Pesticide Residue Exposures in Honey Bees Colonies





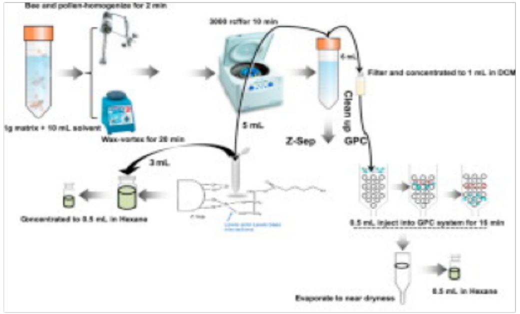
tau-Fluvalinate (LD50 = 15,860 ng/g)

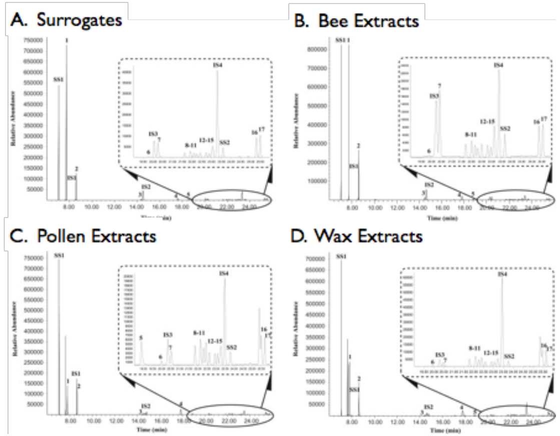
Bee	Pollen	Wax
18 ± 1 ng/g	29 ± 2 ng/g	197 ± 11 ng/g



Coumaphos (LD50 = 46,300 ng/g)

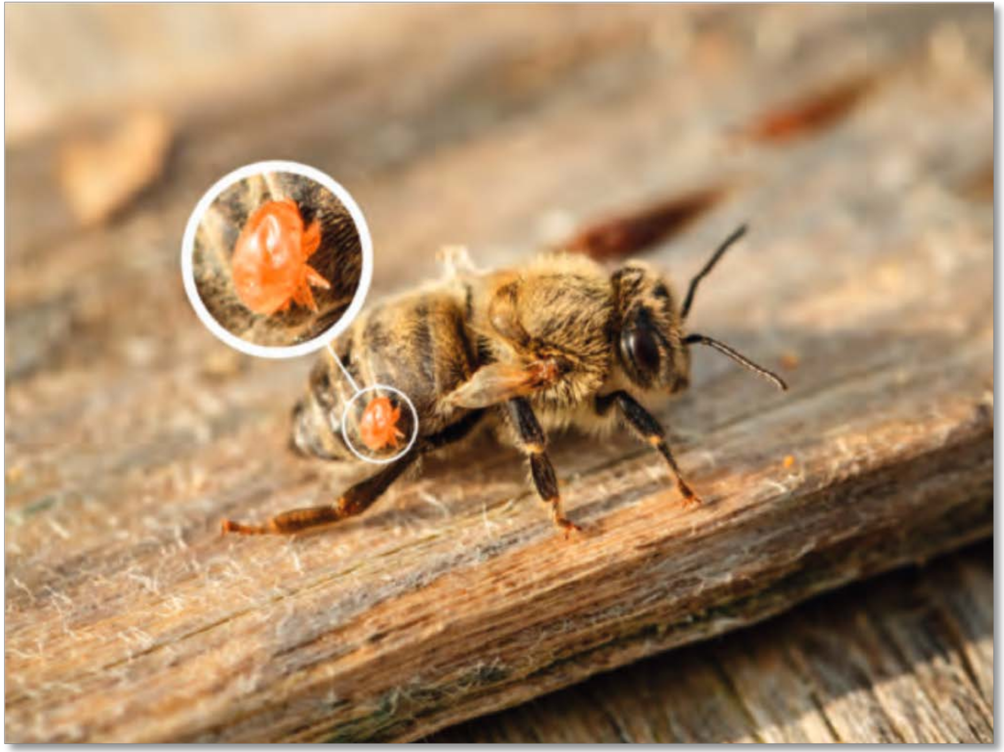
Bee	Pollen	Wax
243 ± 14 ng/g	323 ± 28 ng/g	1100 ± 76 ng/g





Li, Anderson et al. 2015

Major Pest Management Challenge for Apiculture Industry



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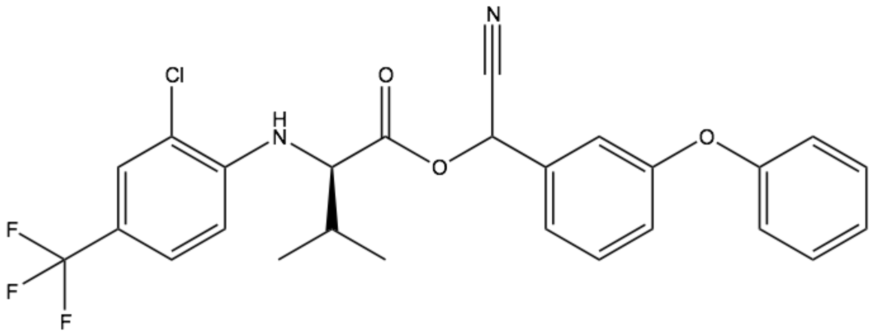
In-Hive Acaricides for Varroa Mite Management



tau-Fluvalinate

Cyano(3-phenoxyphenyl)methyl N-[2-chloro-4-(trifluoromethyl)phenyl]-D-valinate

Pyrethroid Class

VGSC Target Site

1 of 4 Active Diasterioisomers

Section 18 in 1988

kdr Resistance

Bee Nutrition and Immune Health Issues

Reeves, Anderson et al. 2014

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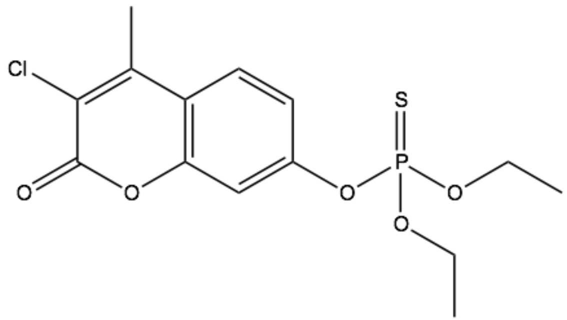
In-Hive Acaricides for Varroa Mite Management



Coumaphos

O-(3-Chloro-4-methyl-2-oxo-2H-chromen-7-yl) O,O-diethyl phosphorothioate

Organophosphate Class

Section 18 in 1999

AChE Target Site

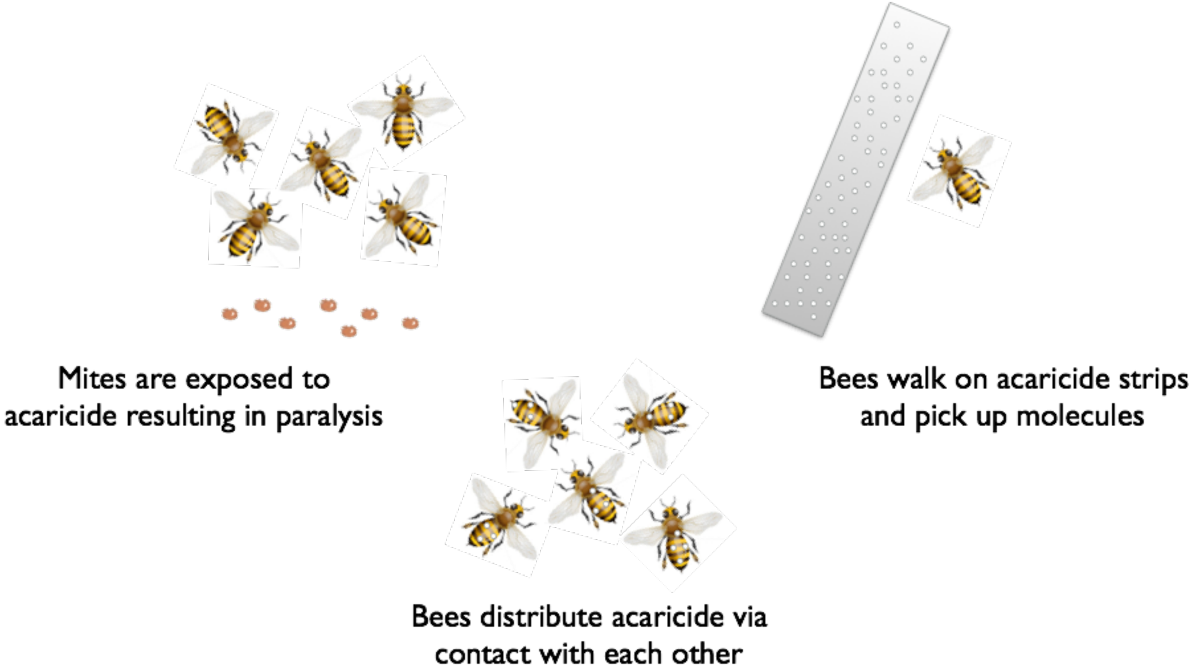
Metabolic Resistance

26+ Commercial Products

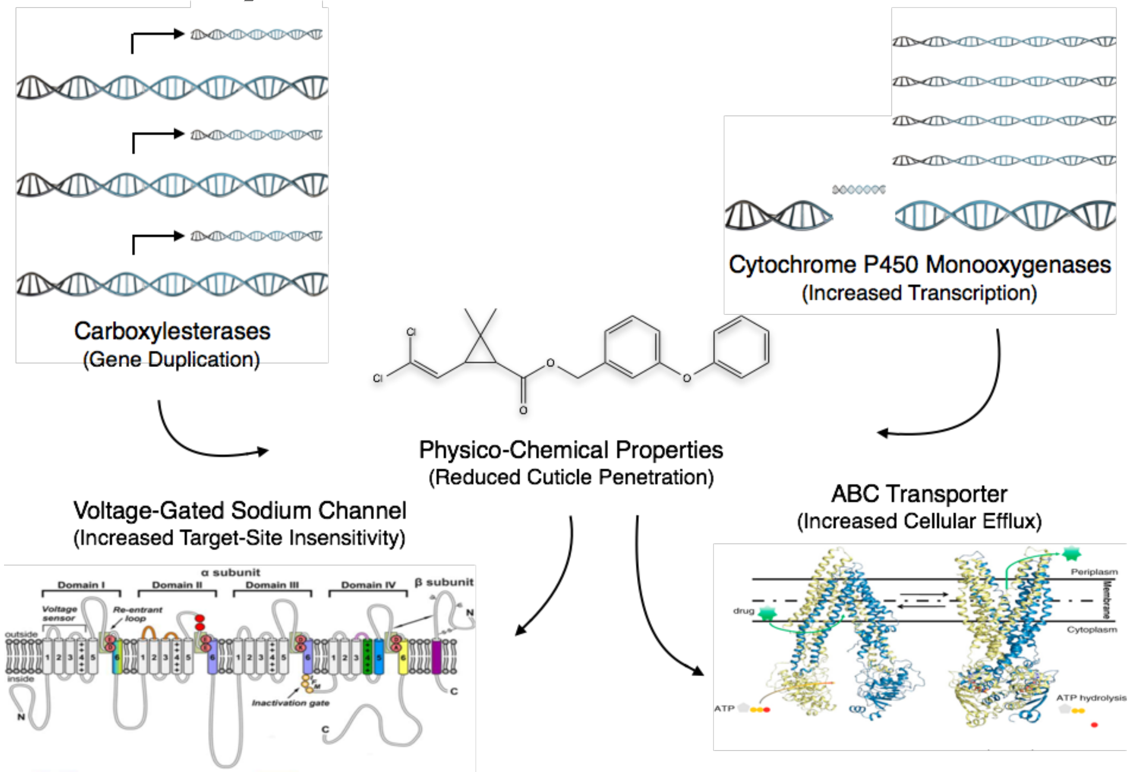
Bee Nutrition and Immune Health Issues

Reeves, Anderson et al. 2014

In-Hive Acaricides for Varroa Mite Management



Metabolic & Target-Site Resistance Limits In-Hive Acaricides Activity



Testing Acaricide Efficacy and Resistance in Honey Bee Colonies

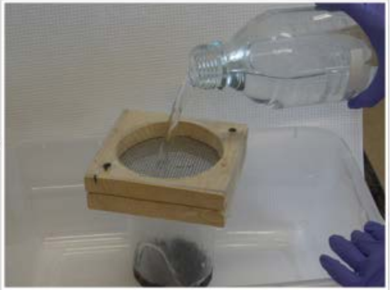
Philene Vu, MS Student

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

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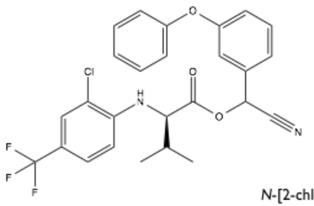
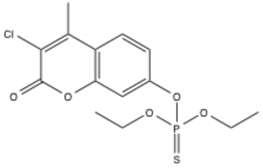
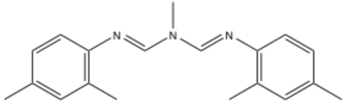
Testing Acaricide Efficacy and Resistance in Honey Bee Colonies



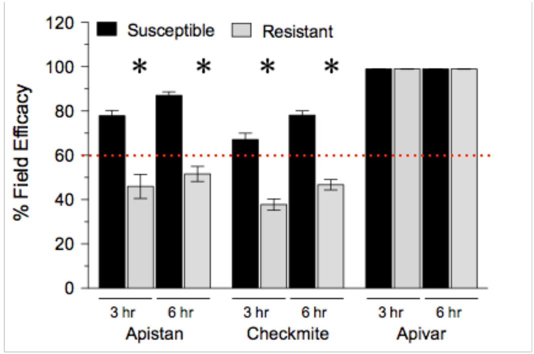
***tau*-Fluvalinate**
(Apistan®, 10.0% ai)
Cyano(3-phenoxyphenyl)methyl
N-[2-chloro-4-(trifluoromethyl)phenyl]-D-valinate

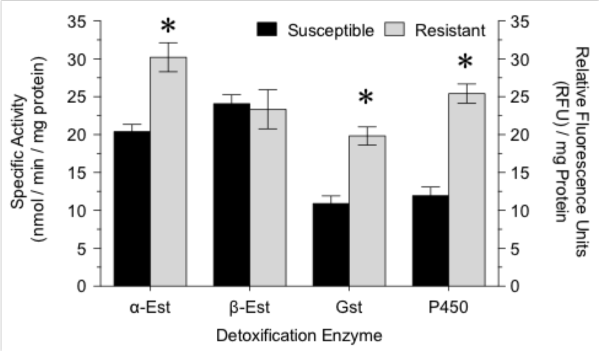


Coumaphos
(CheckMite+™, 10.3% ai)
O-(3-Chloro-4-methyl-2-oxo-2H-chromen-7-yl)
O,O-diethyl phosphorothioate



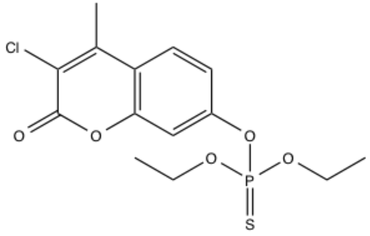
Amitraz (Apivar®, 3.3% ai)
N'-(2,4-Dimethylphenyl)-*N*-{(E)-
[(2,4-dimethylphenyl)imino]methyl}-*N*-methylimidoformamide



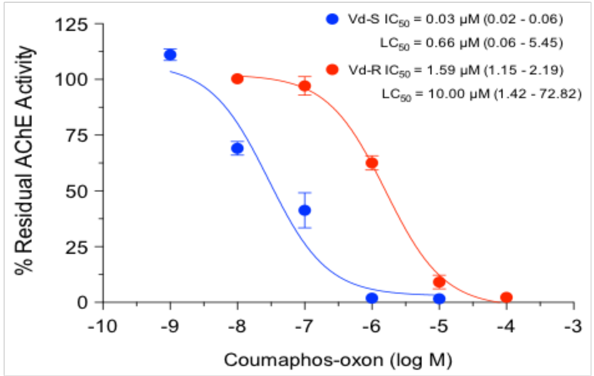


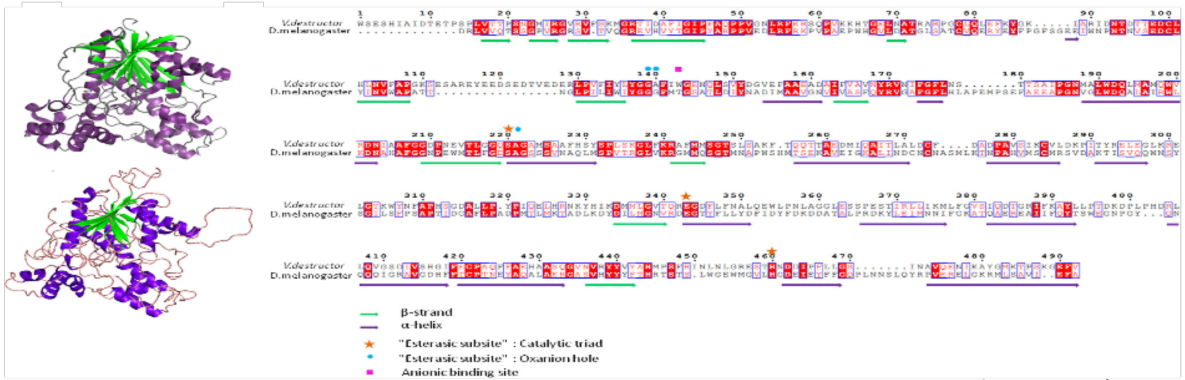
Vu, Anderson et al. 2015

Testing Acaricide Efficacy and Resistance in Honey Bee Colonies



Coumaphos
(CheckMite+™, 10.3% ai)
O-(3-Chloro-4-methyl-2-oxo-2H-chromen-7-yl)
O,O-diethyl phosphorothioate





Vu, Anderson et al. 2015

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Natural Product Stilbenoid Scaffold for Alternative In-Hive Acaricides

Pest Management Science — *Pest Manag Sci* **64**:646–653 (2008)

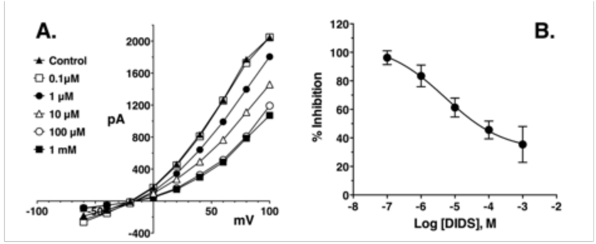
SCI

Nematicidal activity of anion transport blockers against *Meloidogyne incognita*, *Caenorhabditis elegans* and *Heterorhabditis bacteriophora*

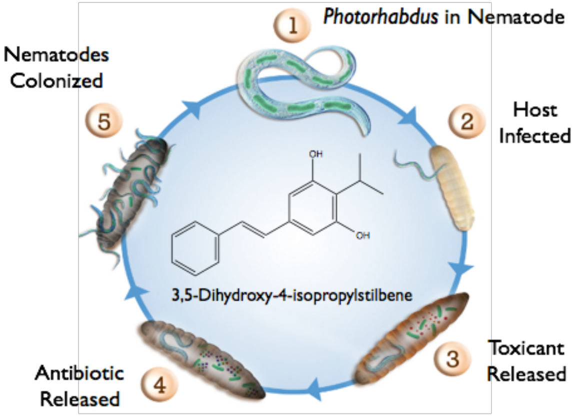
Invert Neurosci
DOI 10.1007/s10158-012-0143-8

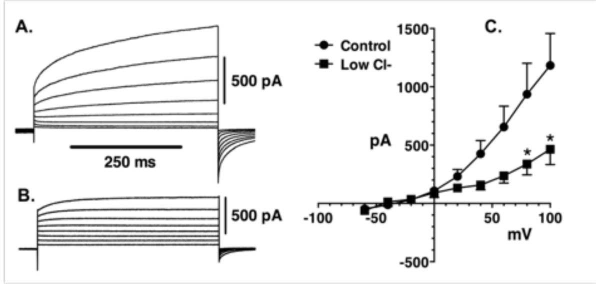
ORIGINAL PAPER

Voltage-sensitive chloride ion channels in *Anopheles gambiae* Sua-1B cells



Electrophysiological effects of DIDS on chloride currents in Sua1B cells. (A) Current-voltage relationship of chloride current in Sua1B cells and inhibition with DIDS (n = 6). (B) Concentration-response curve representing percent inhibition of DIDS at +60 mV (n = 6).





DIDS insensitive chloride currents under planar patch conditions. Traces in (A) and (B) illustrate currents in normal (297 mOsmol) and low (35 mOsmol) chloride extracellular solutions, respectively. Plot (C) shows current-voltage relationship of DIDS-insensitive chloride channels in control and low chloride solutions (n = 3). Asterisks indicate a significant difference in current amplitude at a given voltage step in control or low chloride conditions (unpaired T-test, P < 0.05).

Jenson, Anderson et al. 2015, Jenson, Anderson et al. 2016

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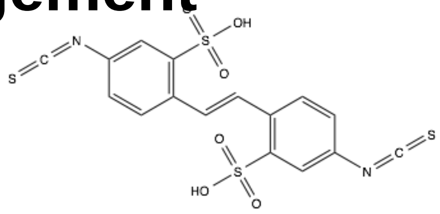
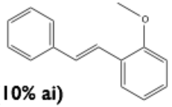
Resistance-Breaking Stilbene Chemistries for Varroa Mite Management



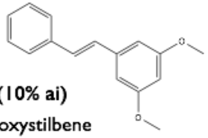
DIDS (10% ai)

4, 4'-Diisothiocyanatostilbene-2, 2'-disulfonic acid



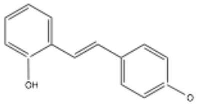
MOSB (10% ai)

2-Methoxystilbene



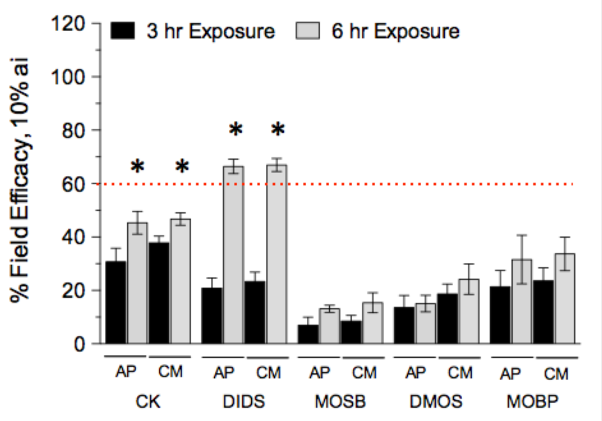
DMOS (10% ai)

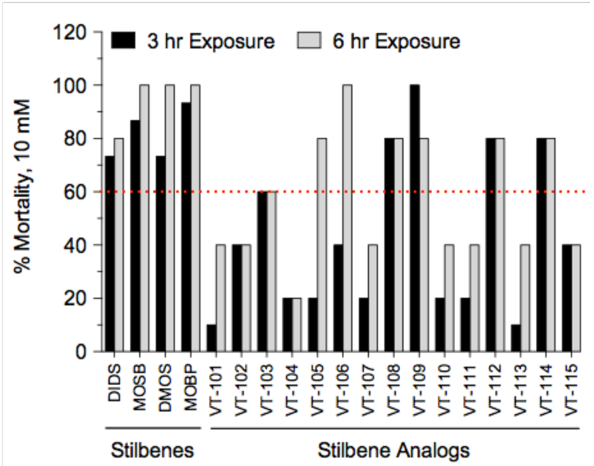
3,5-Dimethoxystilbene



MOBP (10% ai)

(E)-2-(4-methoxystyryl)phenol





Vu, Anderson et al. 2015

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Project Summary & Current Directions

Bee decline has become a nationally recognized problem, demanding attention from both the scientific community and the beekeeping industry.

Pesticide use is one of the primary perceived problems for bee decline, with *tau*-fluvalinate and coumaphos affecting the nutrition and immune health of honey bees (Reeves and Anderson 2014).

Widespread acaricide resistance limits the use of current chemistries to reduce the risk of varroa mite infestations and infectious diseases.

Stilbene chemistries provide an innovative approach for an alternative chemical strategy to deplete or incapacitate varroa mites.

Current research activities are focused on the acaricide-resistance monitoring, identification of metabolic and target-site resistance mechanisms, and discovery of alternative chemistries with acaricidal activity against varroa mites.