Pesticides and Pollinator Decline

John D. Stark
Washington State University

Pesticides and Fish and Wildlife Resources
June 2011

Honey bees are the most economically valuable pollinators of agricultural crops worldwide

Bee pollination involved in 1/3 U.S. diet

Contributes to production of a wide range of fruits, vegetables, tree nuts, forage crops, some field crops, and other specialty crops

Monetary value of honey bees as commercial pollinators in the United States is estimated at about $15-$20 billion annually

Bee colony decline in the United States was first noted in 2006

Scientists named this phenomenon Colony Collapse Disorder (CCD)

Honey bee colony losses are not uncommon

However, losses in recent years differ from past situations because bees are failing to return to the hive (which is largely uncharacteristic of bee behavior)

Bee colony losses have been rapid; colony losses are occurring in large numbers; and the reason(s) for these losses remains largely unknown

USDA concluded in its 2007-2008 progress report (released in June 2009) that “it now seems clear that no single factor alone is responsible for the malady.”

CCD may be “a syndrome caused by many different factors, working in combination or synergistically.”

Three major possibilities

- **pesticides** that may be having unexpected negative effects on honey bees;
- a new **parasite or pathogen** that may be attacking honey bees, such as the parasite *Nosema ceranae or viruses; and*
- **a combination of existing stresses** that may compromise the immune system of bees and disrupt their social system, making colonies more susceptible to disease and collapse.

Stressors could include high levels of infection by the *Varroa mite;* poor nutrition due to apiary overcrowding, pollination of crops with low nutritional value, or pollen or nectar scarcity; exposure to limited or contaminated water supplies; and migratory stress.

U.S. Honey Bee Colony Losses over a five year period, 2007-11

2007/2008 survey = 36% colony loss

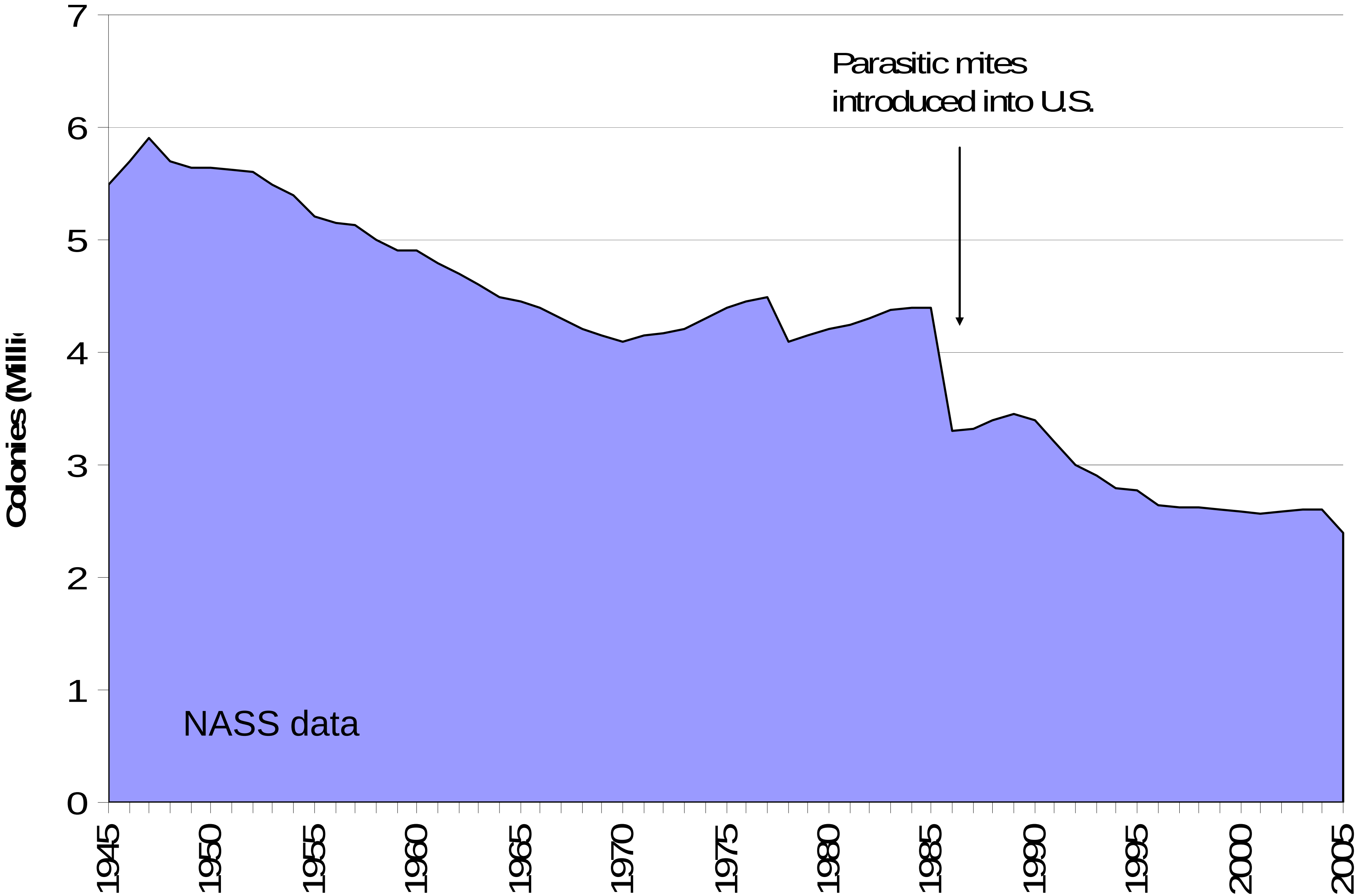
2008/2009 survey = 29% colony loss

2009/2010 survey = 34% colony loss

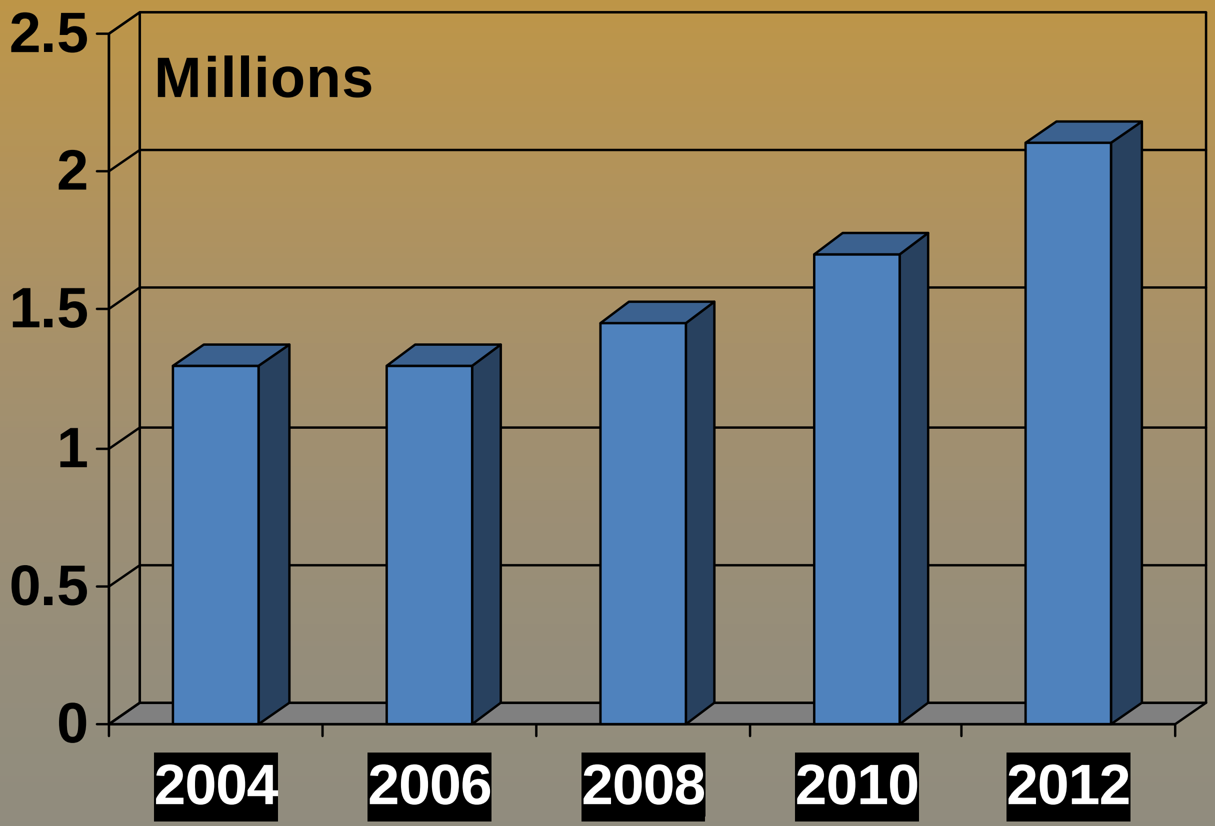
2010/2011 survey = 30% colony loss

vanEngelsdorp et al. 2007, 2008, 2009, 2010, 2011

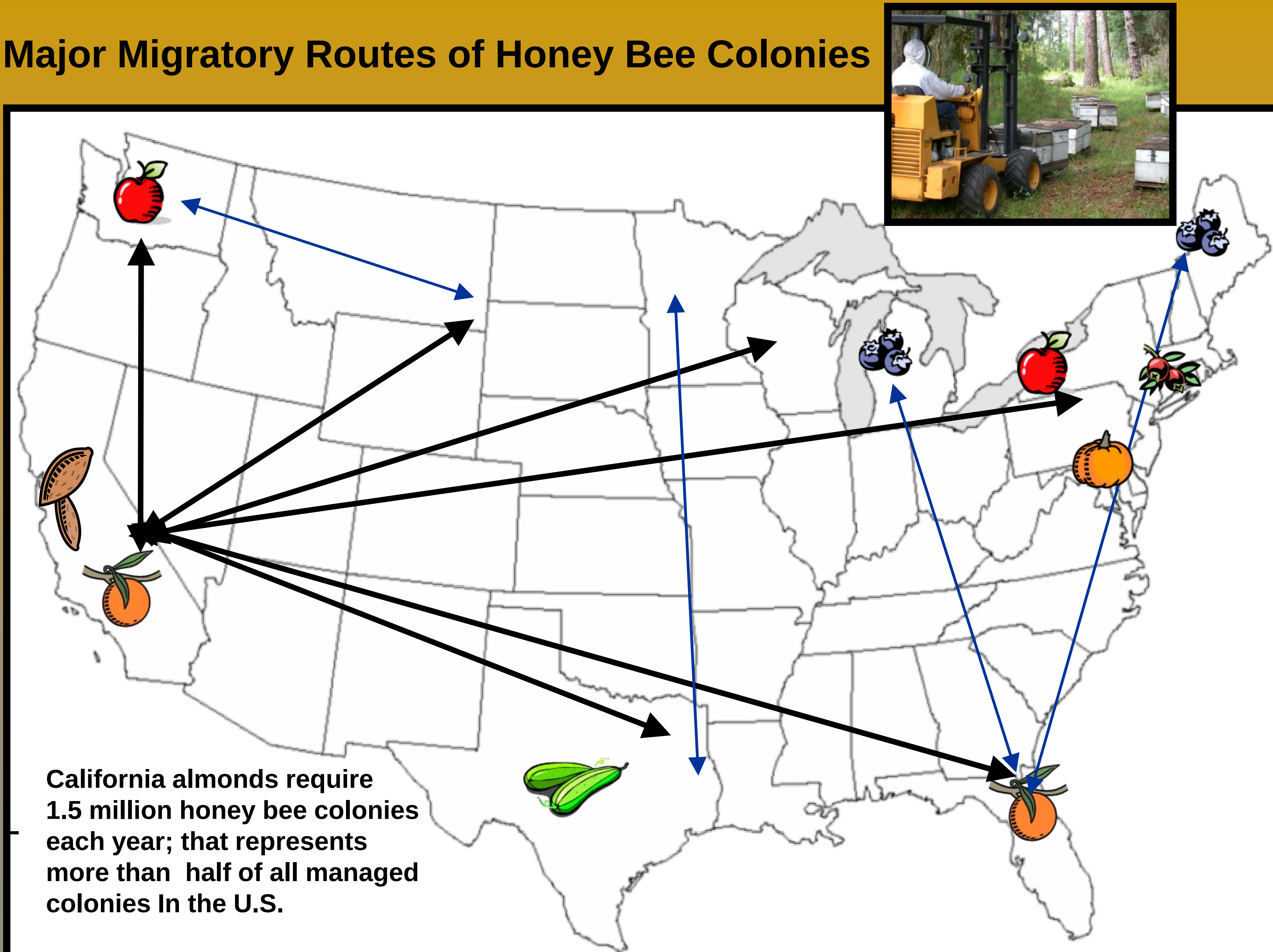
Managed Honey Bee Colonies in the U.S.



Honey bee colonies needed in California almonds

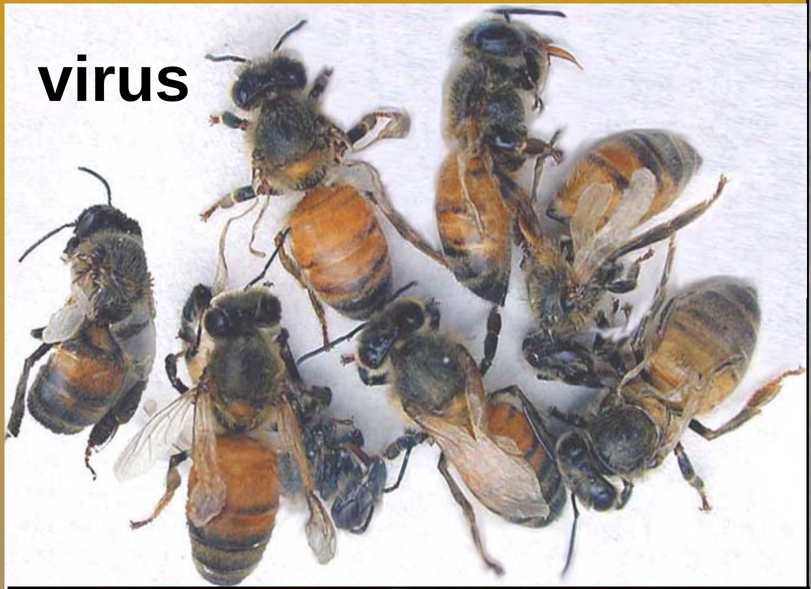


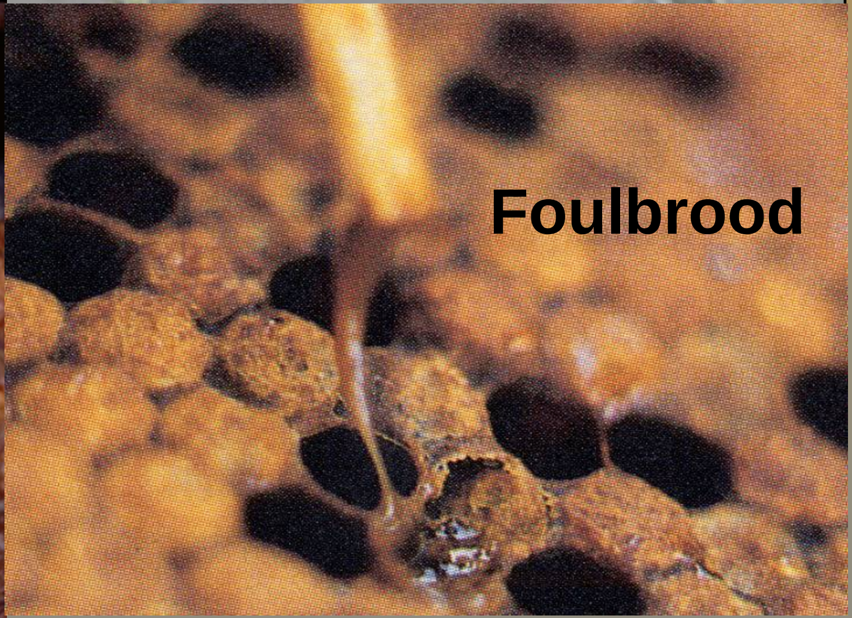
Major Migratory Routes of Honey Bee Colonies



California almonds require 1.5 million honey bee colonies each year; that represents more than half of all managed colonies In the U.S.

Honey Bee Diseases and Pests





Colony Collapse Disorder (CCD)

Rapid loss of adult worker bees

Few or no dead bees in colony

Colonies dead with excess brood

Small cluster with queen present

(vanEngelsdorp, Pettis et al. PLoS One 2009)

Working Hypothesis

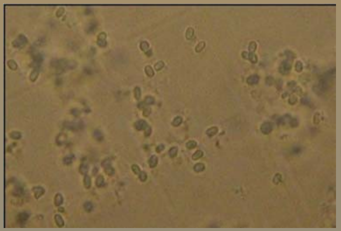
Primary Stress

Varroa Mites

Management
Nutrition
Pesticides

Secondary Pathogens

Viruses



Nosema

Fungi


Stress Interactions = Decreased Longevity

Causes of Colony Losses = Multiple Stressors

Transporting Colonies

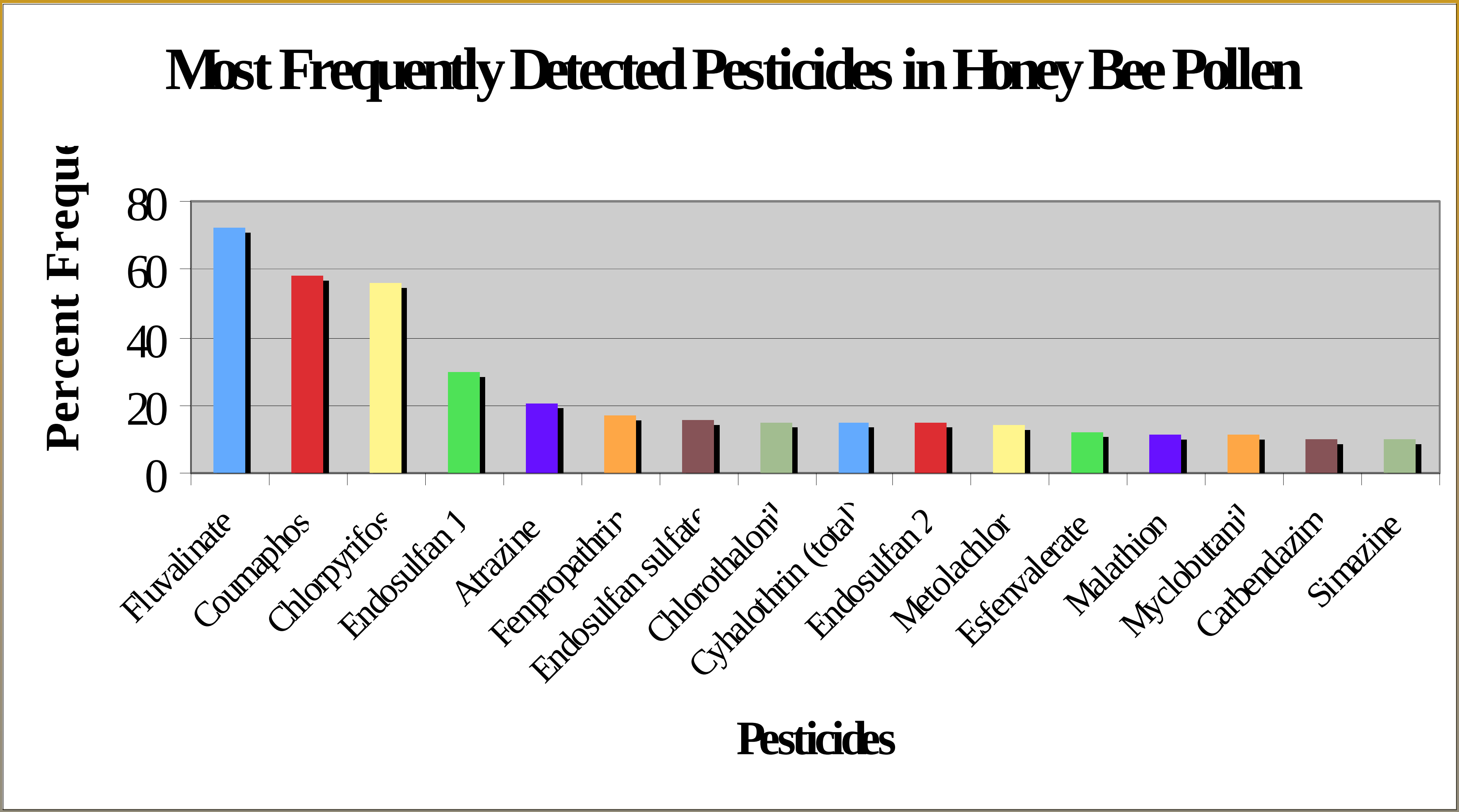
* Loss of Bee Forage = nutrition

* Pesticides

* Varroa

Pathogens

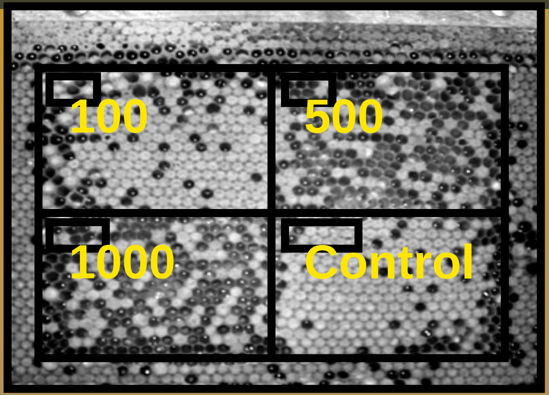
Toxins



Mullen et al. PLoSOne 2010

Coumaphos impregnated wax and effects on worker survival

Created wax foundation with three levels of coumaphos in the wax

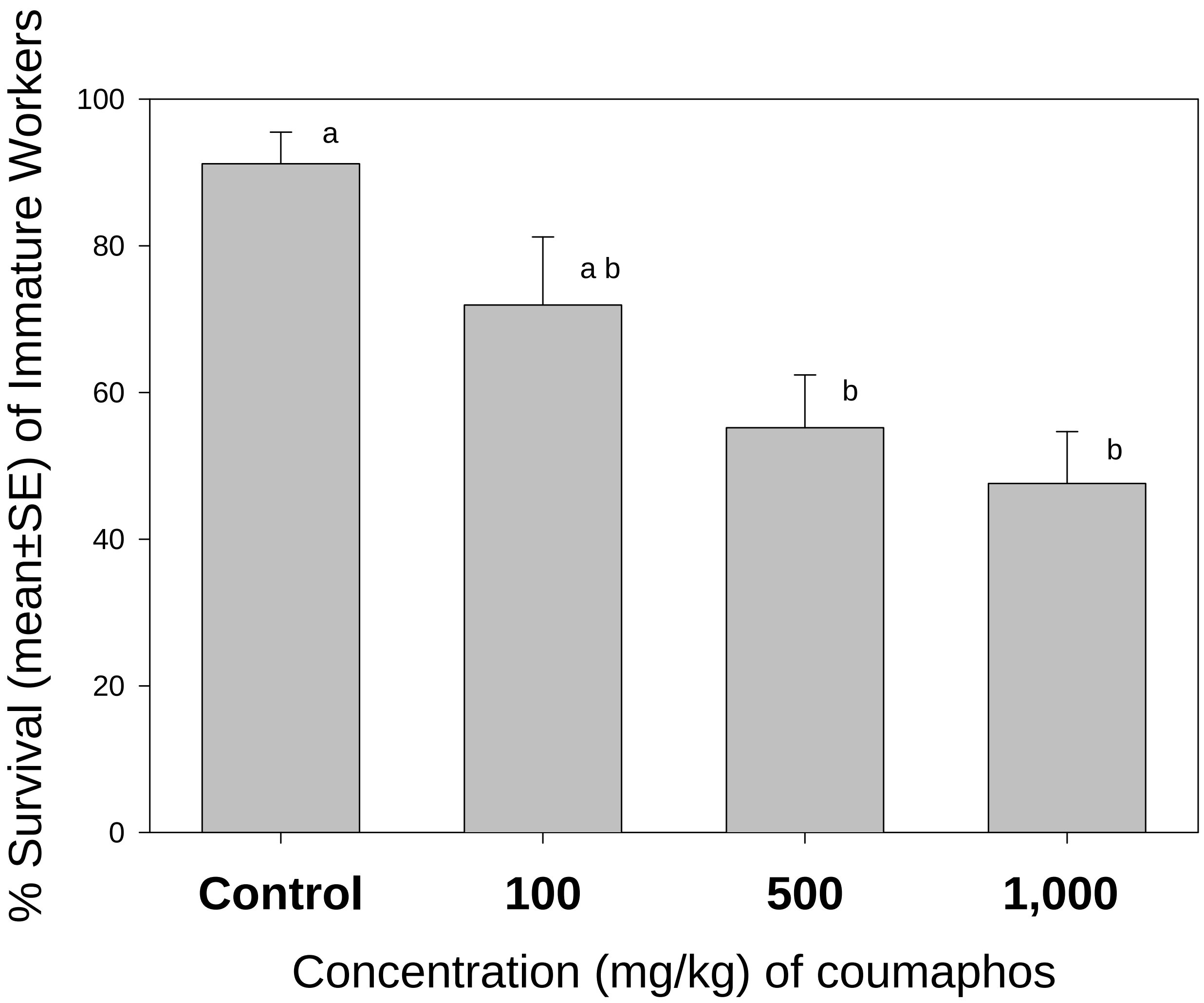


Placed in separate colonies to draw out comb

Combined 4 pieces of drawn comb into a single 4-way comb for queens to lay in

Placed 4-way combs in 10 hives and monitored egg laying and worker survival

Survival of worker brood from 4-way comb



Pesticides (two sources)

1. Beekeeper
   > *for mite controls*

2. Environment
   > *crop protection, mosquito control*

Possible pesticide-pathogen interaction

Expose bees to imidacloprid at low levels

(sub-lethal)

(Univ. of Maryland)

Challenge with Nosema

Determine Nosema infection rates

Possible pesticide-pathogen interaction

Imidacloprid fed in MegaBee protein patty
over 10 weeks to full size honey bee colonies

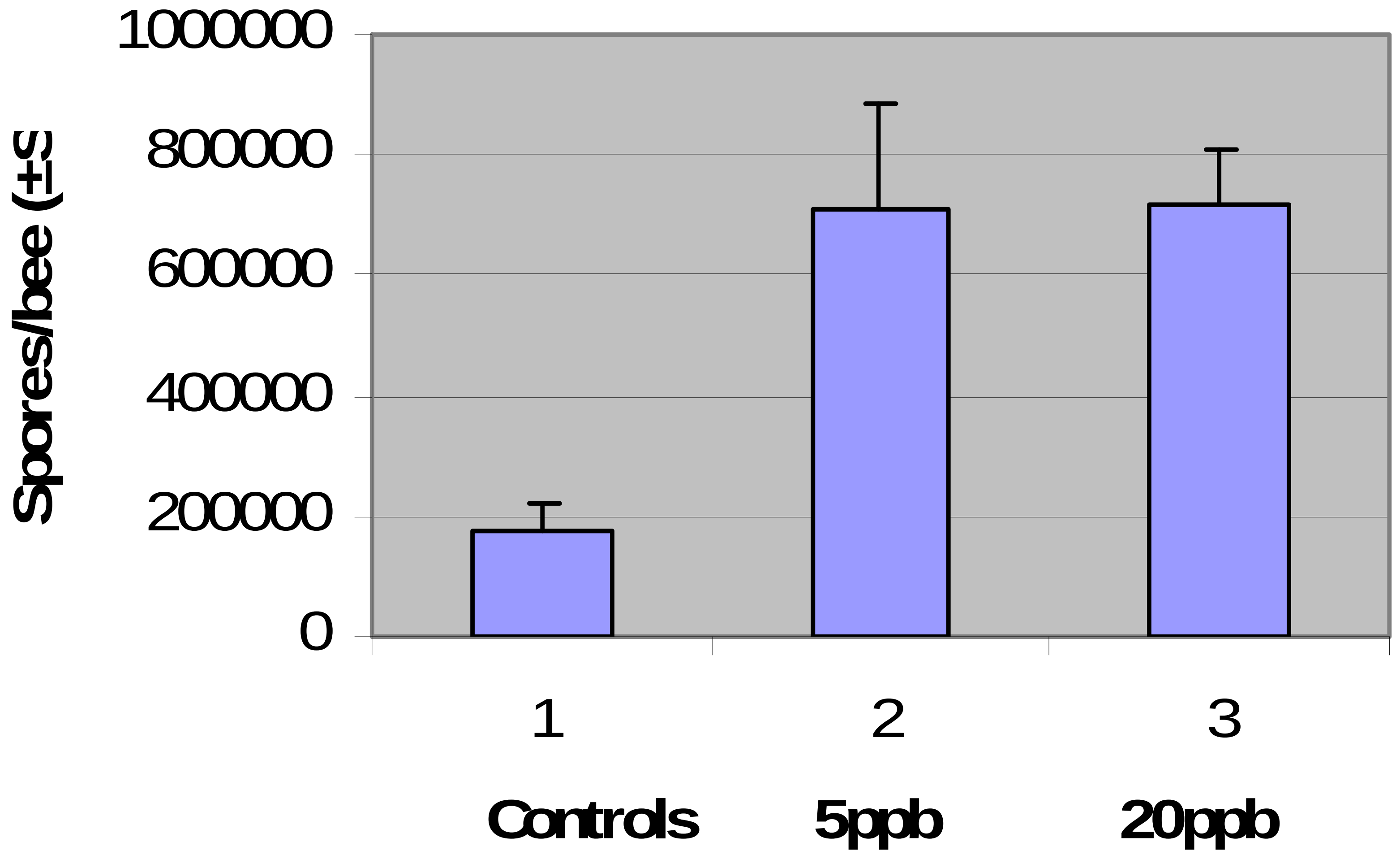
Control	5 ppb	20 ppb
N = 10	N = 10	N = 10

Emerging brood combs pulled from 4 colonies
of each treatment group at week 6 of exposure

Emerged bees fed sucrose with ca. 250,000 spores
per bee over a two day period in laboratory cages

Bees removed on day 12 and Nosema counts
performed on individual bees (n=10 / cage).

Nosema Spores / bee (12 days old)



Pollinator Health Summary

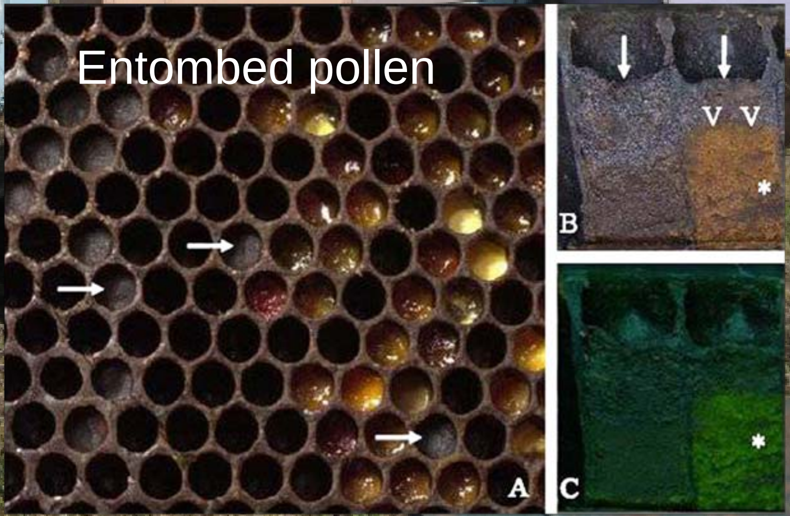
- All pollinators are threatened
- Declining honey bee health is complex
- Demonstrated effects with miticides and Agricultural chemicals
- Pesticides/Pathogen interactions



Entombed pollen associated with increased colony death
Fungicide chlorothalonil was at elevated levels in entombed pollen
vanEngelsdorp et al. *J. Invert. Pathol*. **2009**

2010 Study in France showed interaction with imidacloprid and Nosema

Beekeepers ?



Bees and Pollinators

need diversity of floral resources

A honey bee hive and organic vegetable garden on the White House lawn, thanks to Michele Obama

A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bee

James E. Cresswell Ecotoxicology 2010

•Meta-analysis 14 published studies on effects of imidacloprid on honey bees under laboratory and semi-field conditions

•Results - imidacloprid at field-realistic levels in nectar will have no lethal effects, but will reduce expected performance in honey bees by between 6 and 20%

• Statistical power analysis showed that published field trials that have reported no effects on honey bees from neonicotinoids were incapable of detecting these predicted sublethal effects with conventionally accepted levels of certainty

•These findings raise renewed concern about the impact on honey bees of dietary imidacloprid, but because questions remain over the environmental relevance of predominantly laboratory-based results future studies are required

Sub-lethal effects of pesticide residues in brood comb on worker bees

Judy Yu Wu
Washington State University
Department of Entomology
Master's Thesis

Research objectives

Pesticide load survey	level of exposure in the "field," correlation between failing colonies & pesticide load?
"Life-table" study	development & survivability (egg to adult)
Cage longevity tests	adult lifespan
Susceptibility experiment	susceptibility to other pests Nosema

Pesticide load survey

5 categories of samples (n_{total}=98):

1) PNW migratory colonies (poor) (n=24)

2) CRC (collaborative research colonies) (n=24)

3) QPO queen producing operations (n=19)

4) FWS commercial foundation wax (n=7)

5) suspect "CCD" (Dr. Jeff Pettis USDA, MD)

(n=24)

Chemical analysis:

- Samples sent to Roger Simonds at the USDA- AMS lab in Raleigh N.C.

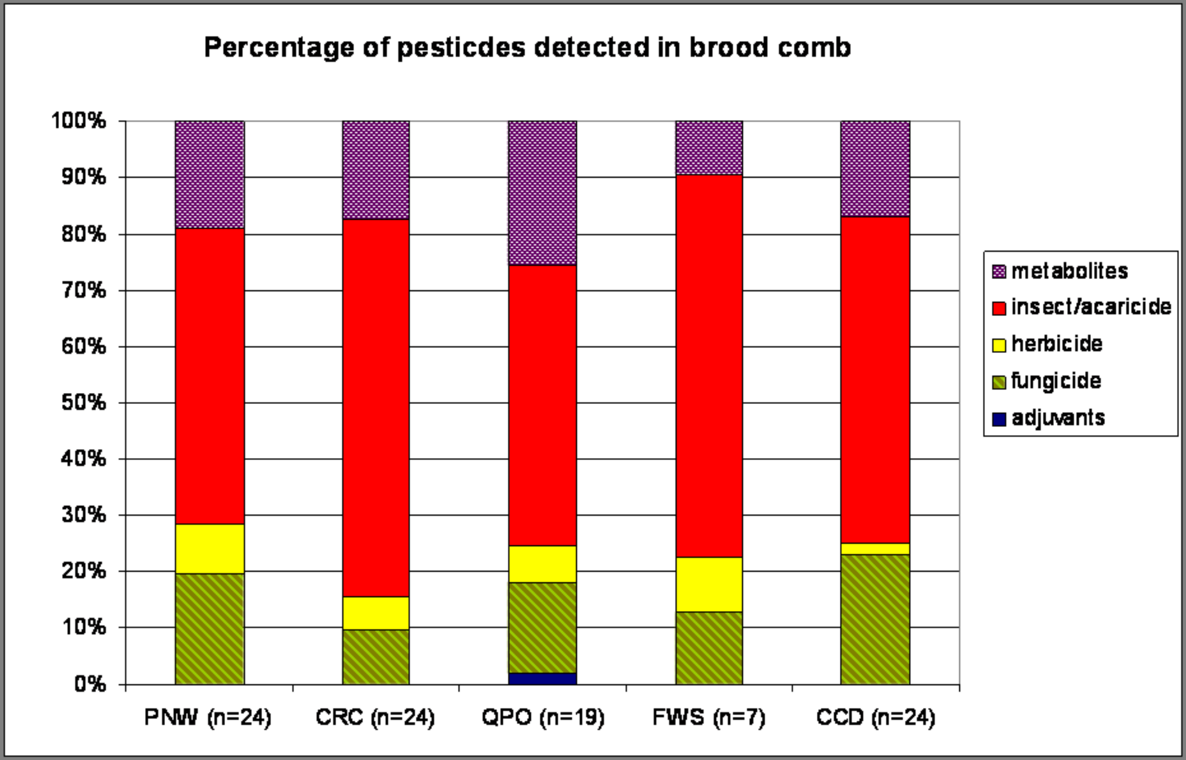
- 171 most commonly used pesticides tested
  (including coumaphos, fluvalinate, & amitraz metabolite (DMPF)).

- Residues in combs determined with GC/MS.

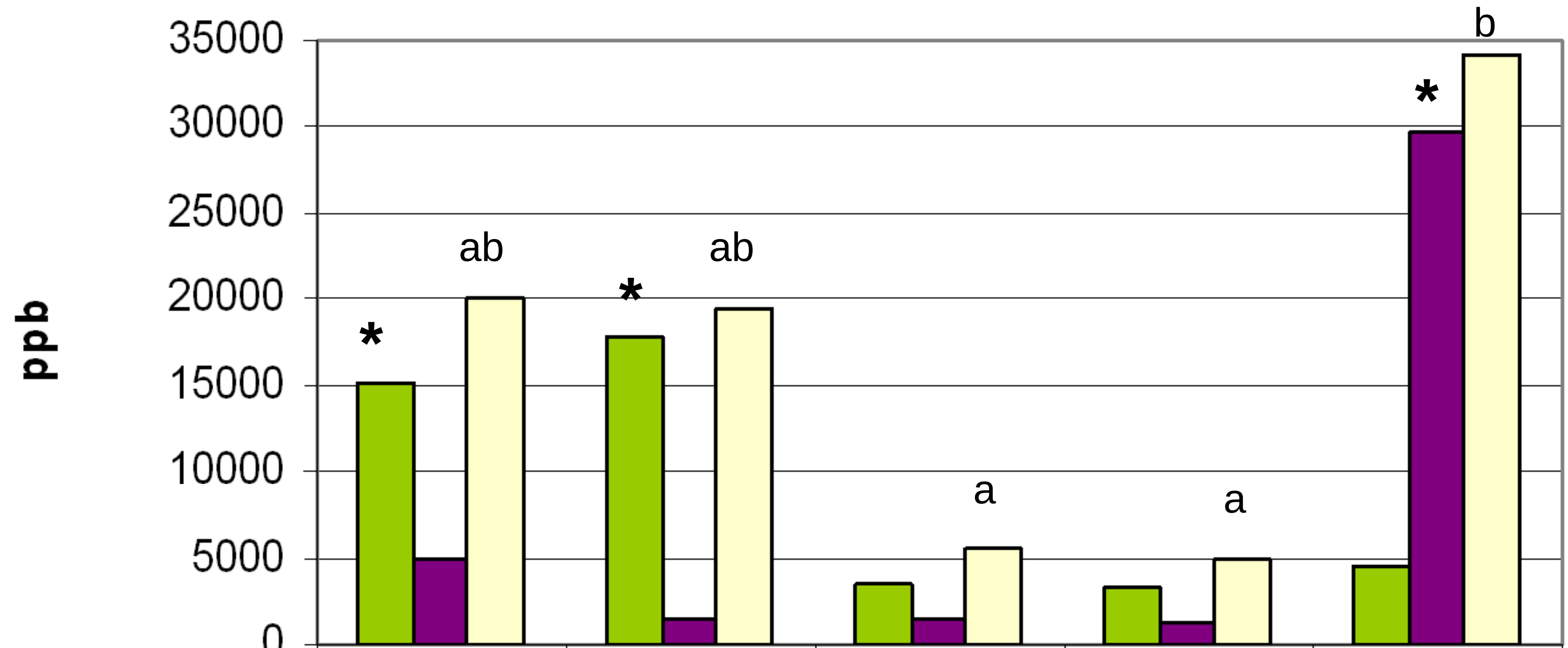
What did we find?

Out of 171 screened, we found **66** different pesticides, **10** metabolites, and **1** synergist (piperonyl butoxide) = **77**

Graph 1. Organization of pesticide groups detected in five categories of honey bee brood comb (PNW= migratory colonies in Pacific northwest, CRC= collaborative research colonies embedded in a migratory operation, QPO=California queen producing operation, FWS= commercial foundation wax sheets, CCD= colonies suspected to have dead from colony collapse disorder).

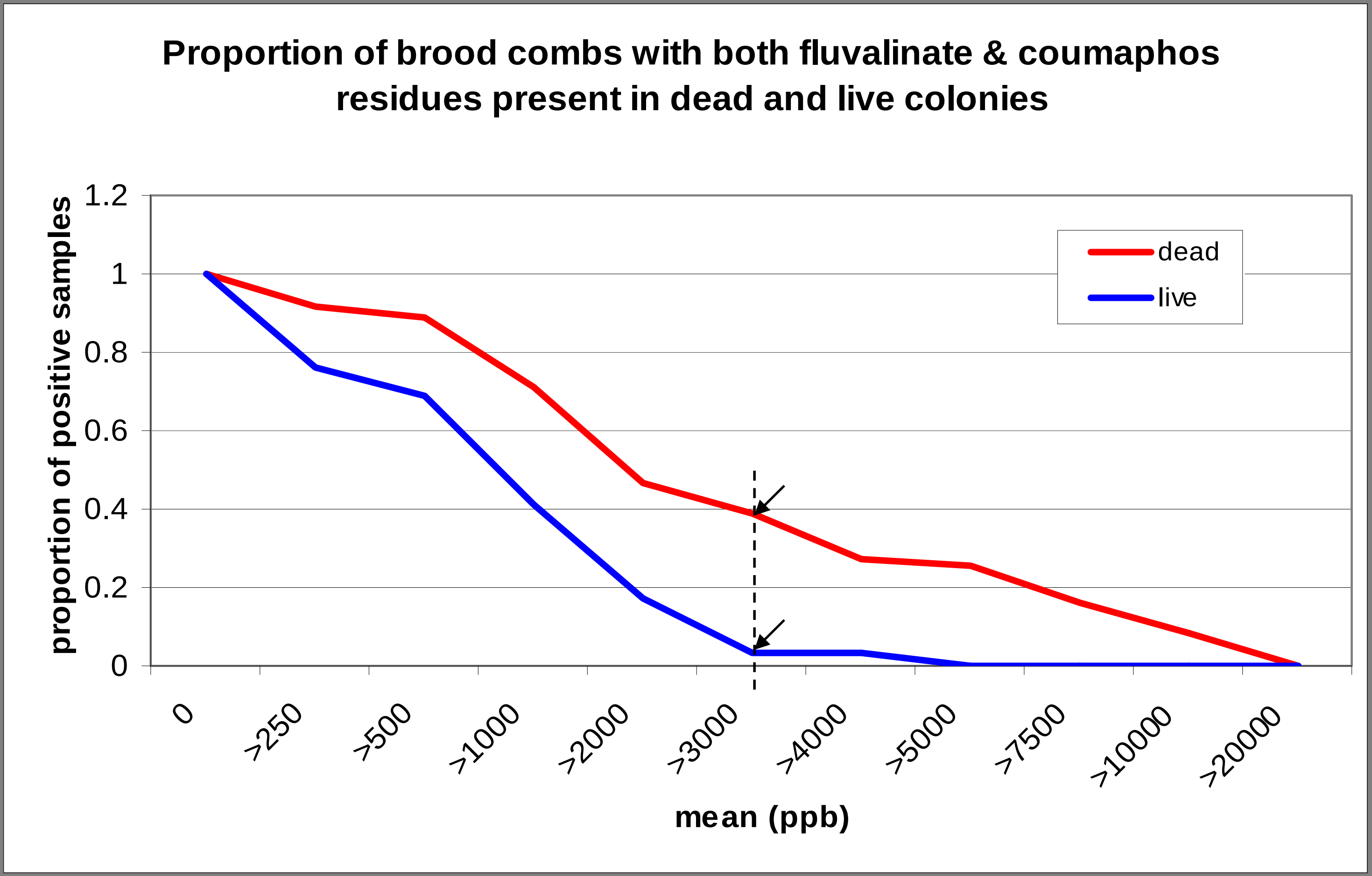


Graph 2. Mean insect/acaricide, fluvalinate, and coumaphos residue levels detected in five categories of brood comb (* and letters denote significance).



	PNW (n=24)	CRC (n=24)	QPO (n=19)	FWS (n=7)	CCD (n=24)
fluvalinate	15202	17907	3457	3357	4428
coumaphos	4863	1407	1488	1243	29730
Insect/Acaricide	20123	19479	5506	4890	34172

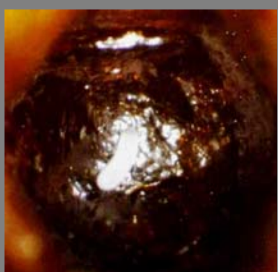
Graph 4. The number of samples with concurrent detections of fluvalinate & coumaphos residues at various levels. (34% of samples from dead colonies and 3% of from live colonies had fluvalinate and coumaphos levels at 3,500 ppb).



“Life table” study

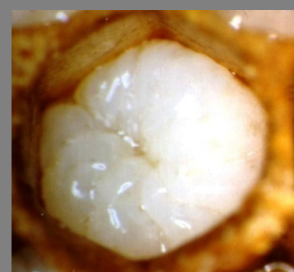
- Enclosed queen lays for 24 hrs on either block
  (up to 1000 eggs daily)

- After 24 hrs. development of brood recorded at:



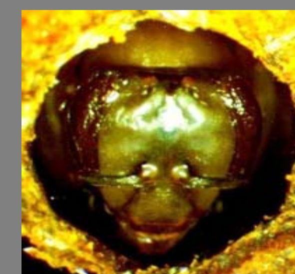
day 1 (egg)

day 4 (1st instar)



day 8 (5th instar)

day 12
(capped pupae)



day 19
(before incubation)

(Capped brood incubated (34°C))

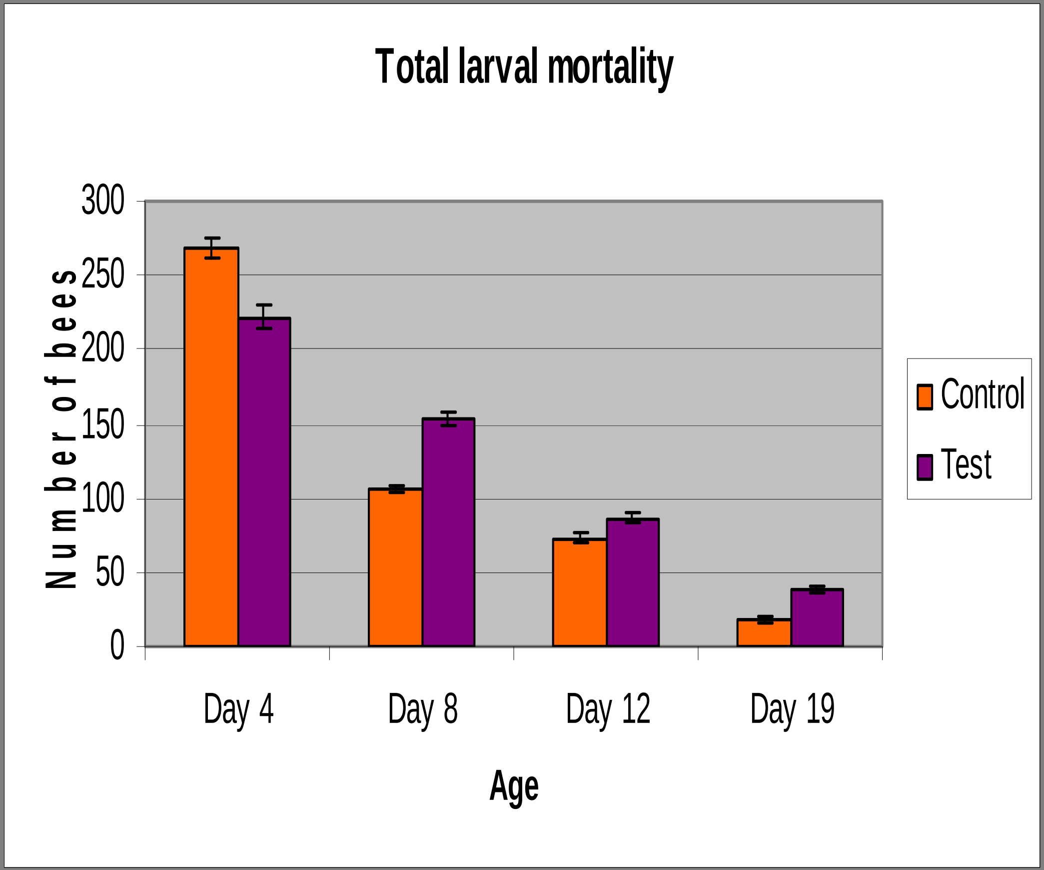
Newly emerged adults

1) cage longevity tests

2) behavioral study

*3) susceptibility expt
(collaboration with M. Smart)

“Life table”



Total of 25 paired treatments (’08-’09)

Stats for 2009 *in progress*

2008 survivorship:
control 79%, test 70%

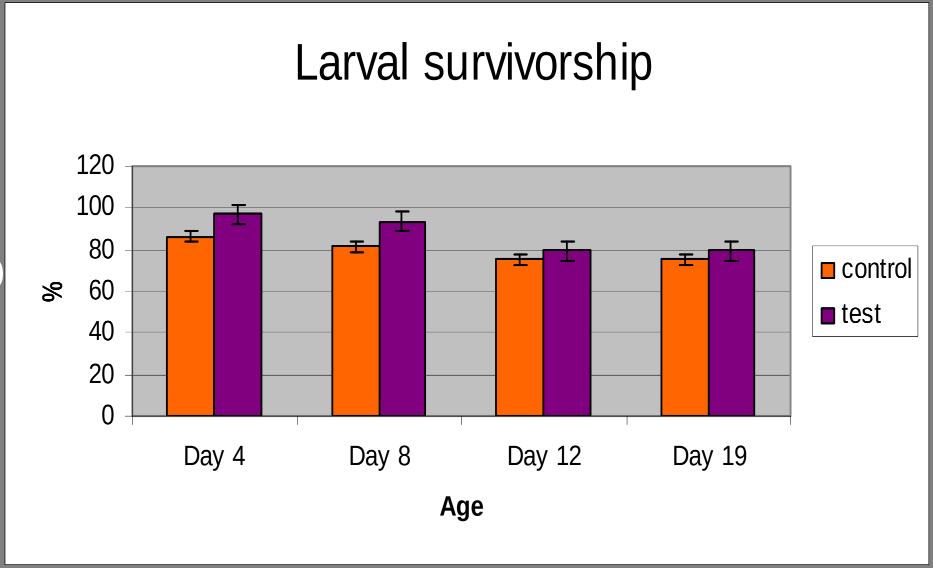
Larvae reared on combs without residues had greatest survivorship 95%

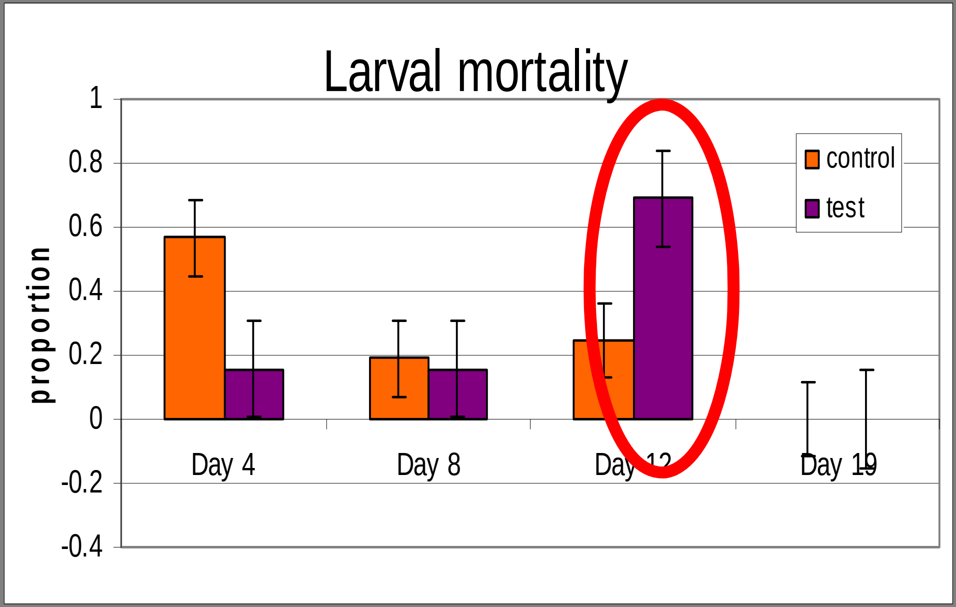
Larval mortality was higher for test bees on day 8 and 12 of development

Larvae reared on test combs with 17 diff. pesticides & high levels of coumaphos expressed delayed development

Frame # 5

Control comb was made just prior to starting expt. (comb age effect)





Test comb:

- 17 pesticides

All but 2 are toxic to bees !

Highly toxic

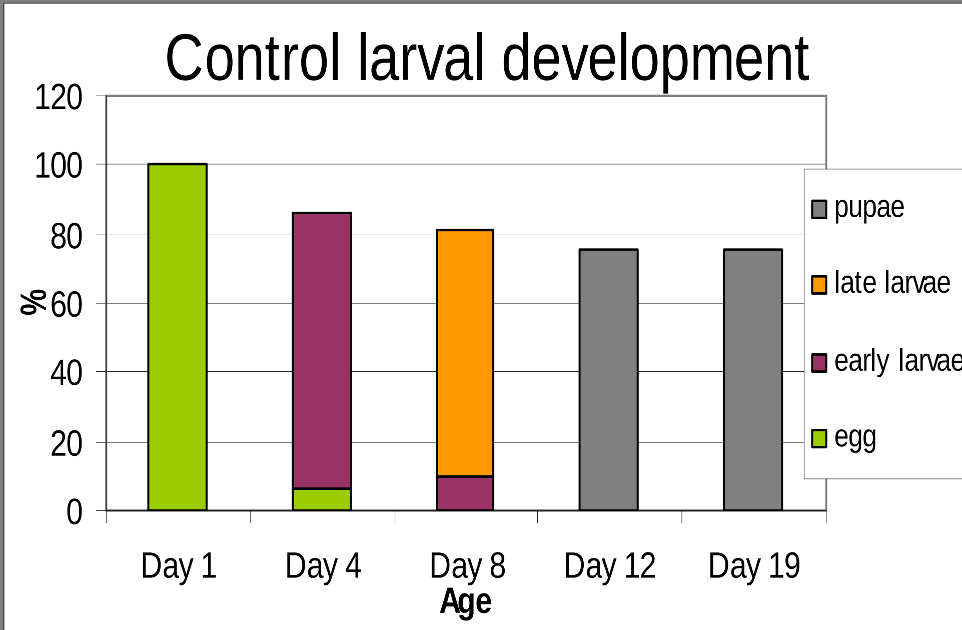
Mod. toxic

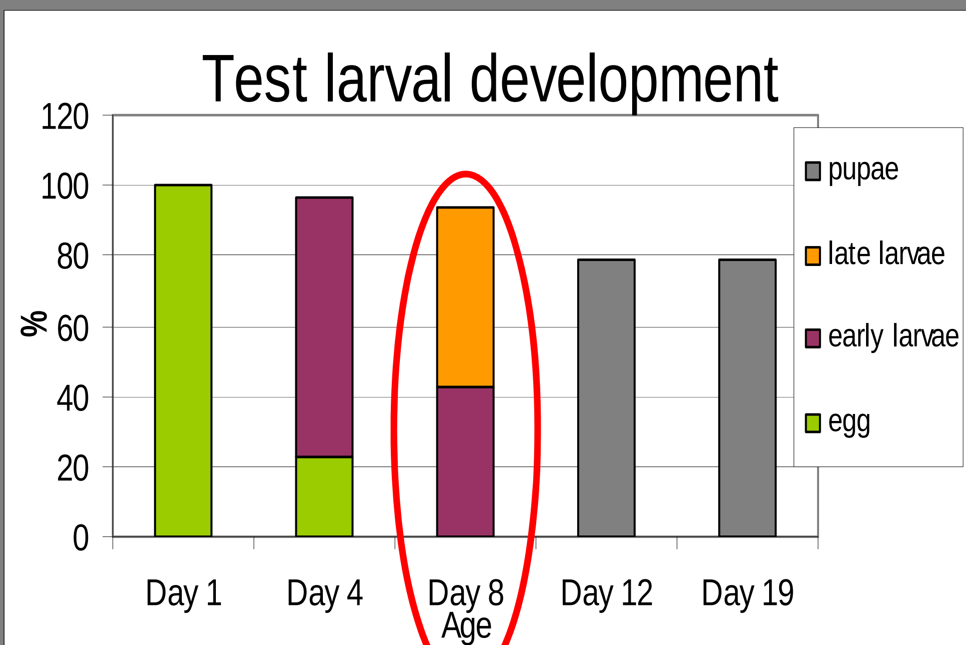
Control

Pesticide	ppb
Coumaphos	**20.9**

Test

Pesticides	ppb
3-hydroxy-carbofuran	**23**
Aldicarb	**20**
Carbofuran	**32**
Chlorothalonil	4
Clothianidin	**35**
Coumaphos	**22100**
Coumaphos Oxon	**1850**
Cyfluthrin	**7.9**
Dinotefuran	**97**
Diphenylamine	281
Endosulfan 1	**1**
Fluvalinate	**164**
Imidacloprid	**45**
Malathion Oxon	**22**
Oxamyl	**22**
Thiacloprid	**113**
Thiamethoxam	**38**





Control

Pesticide	ppb
Coumaphos	**20.9**

Test

Pesticides	ppb
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Chlorothalonil	4
Clothianidin	**35**
Coumaphos	**22100**
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Fluvalinate	**164**
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Oxamyl	**22**
Thiacloprid	**113**
Thiamethoxam	**38**

2 Fungicides

3 Carbamates
+ 1 meta

1 OP + 2 meta.

1 Cyclodiene

2 Pyrethroids

5 Neonicotinoids

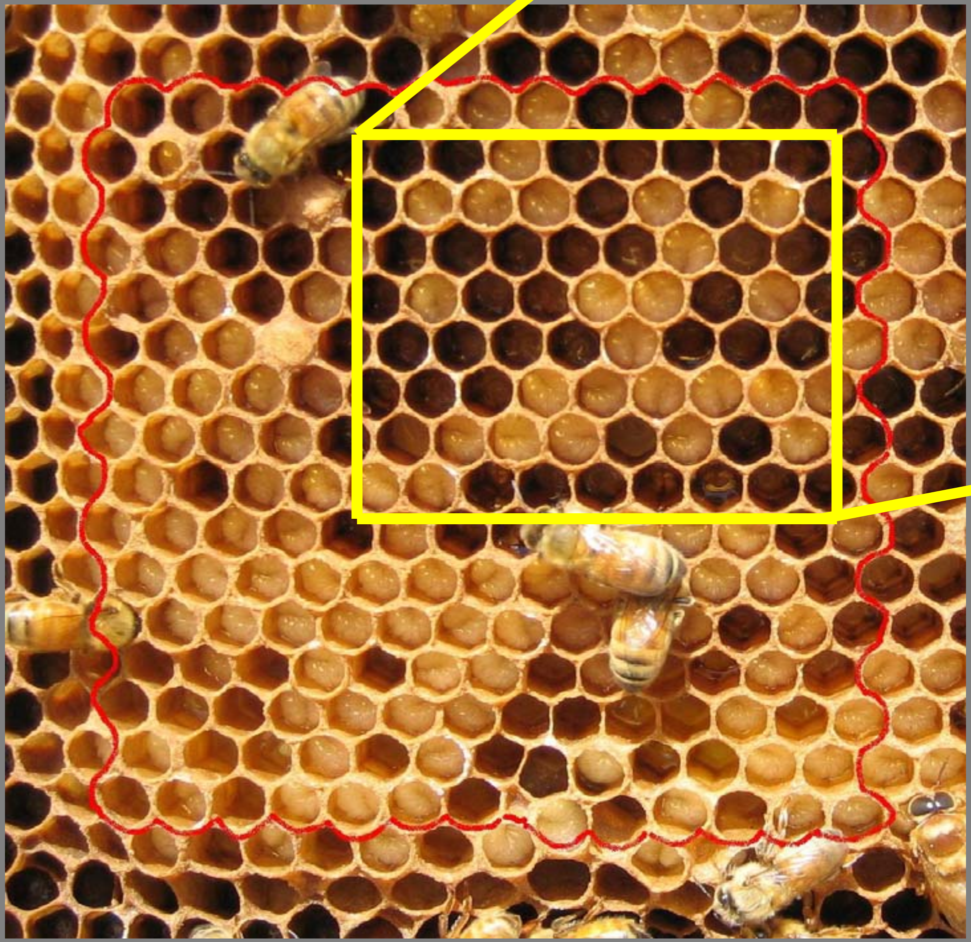
Total = 17

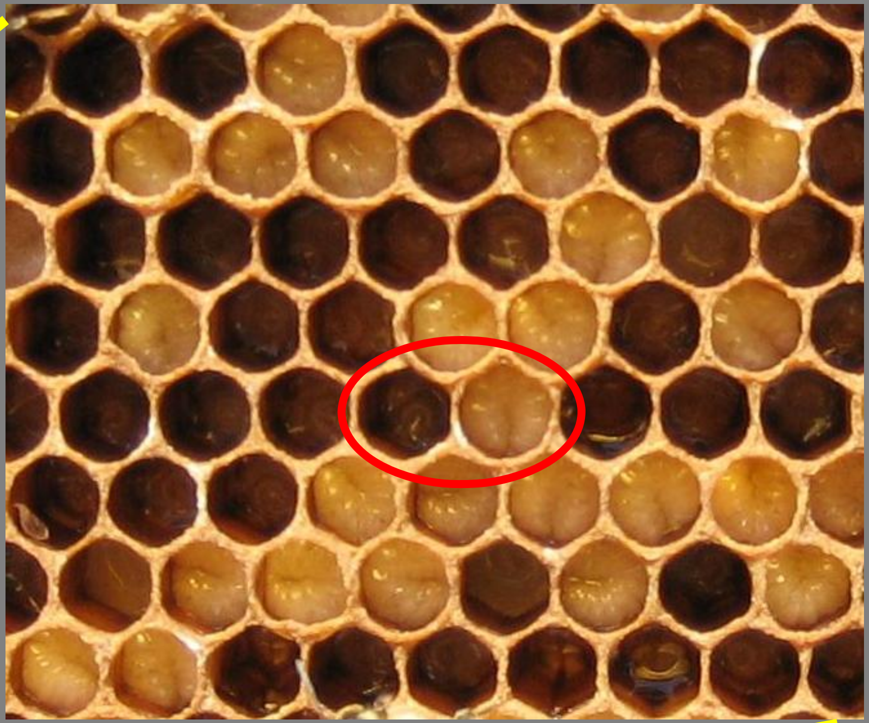
highly toxic

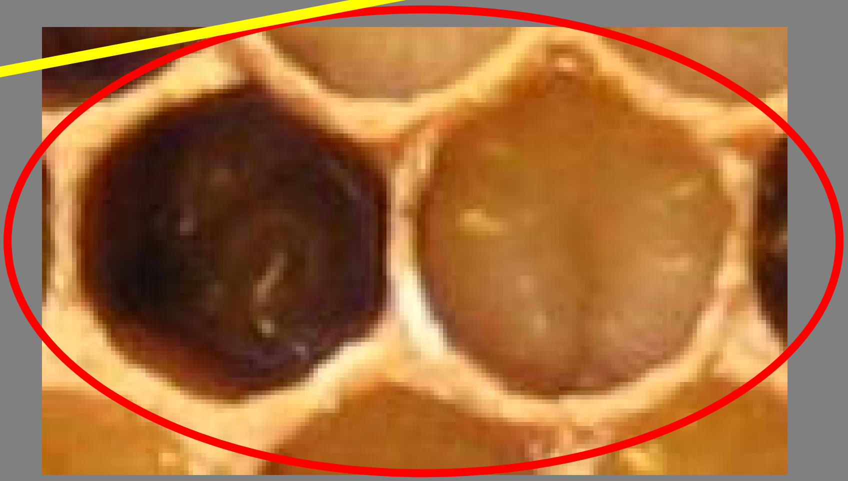
mod. toxic

Frame # 5

Test comb: day 8



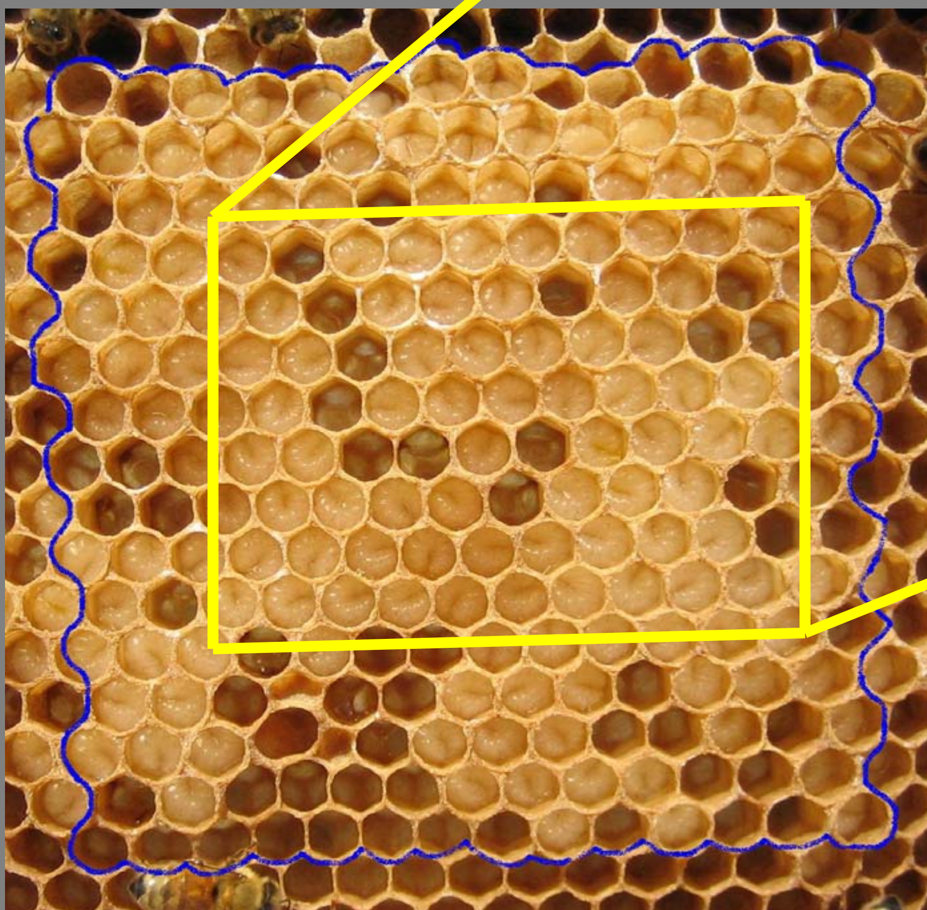


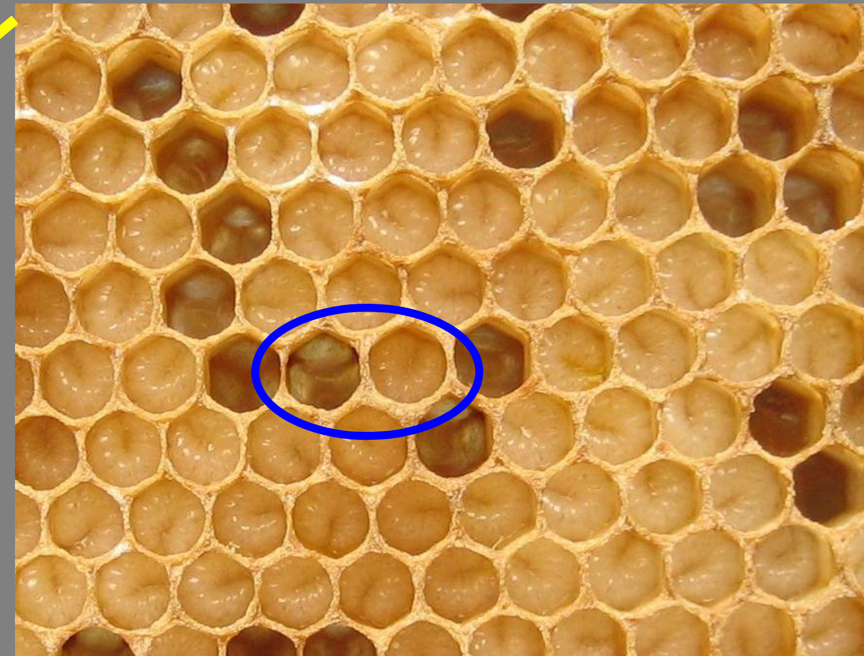


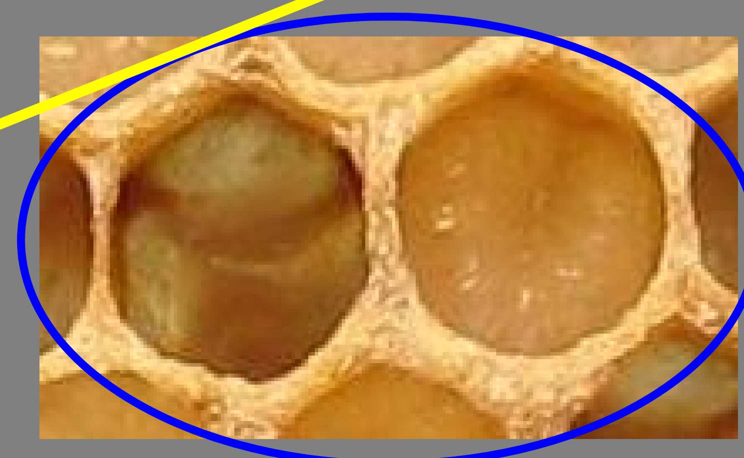
Delayed development   Normal development

Frame # 5

Control comb: day 8







Cage longevity

Newly emerged adults were counted, color-tagged, and separated by treatments

Cages were kept in incubators (30°C) without light

Mortality recorded daily

Gravity feeders:

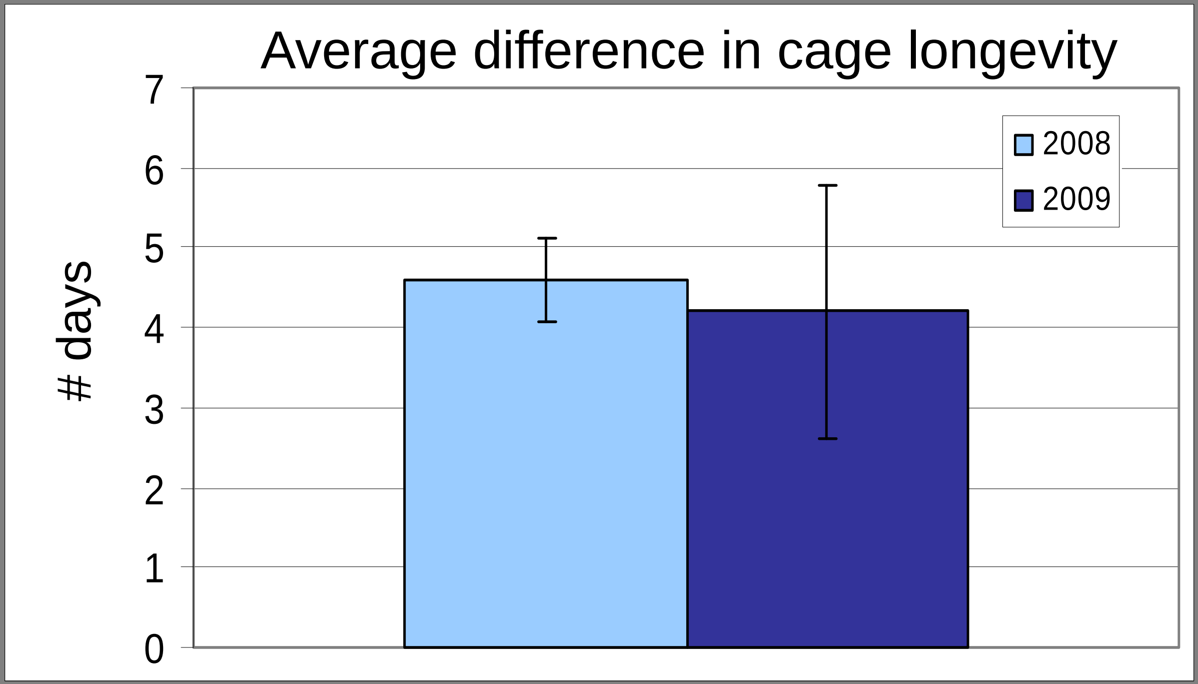
50:50 sugar solution & water

Testor’s enamel

Cage longevity results

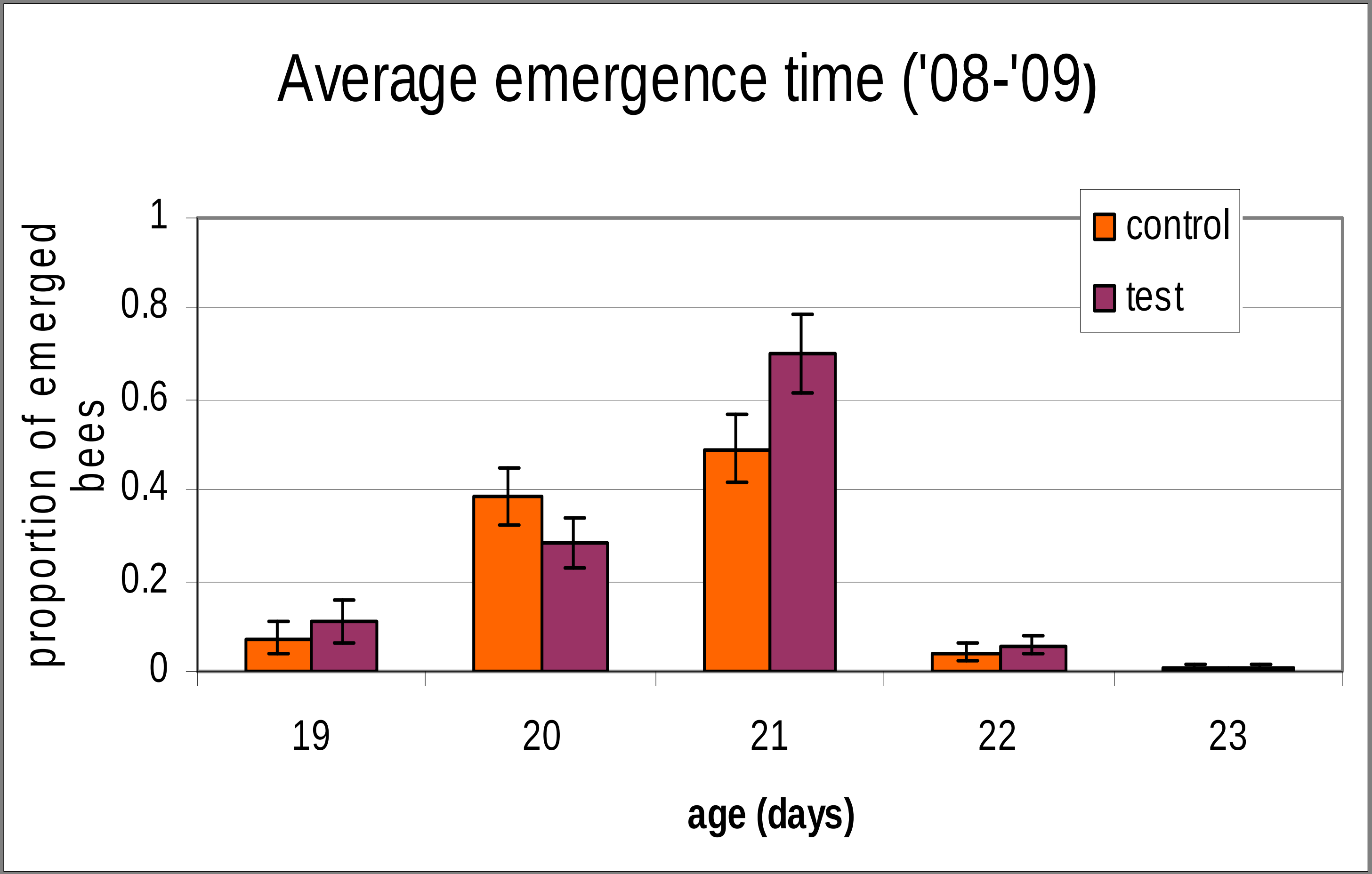
2008: Adult control bees generally outlived adult test bees in the cage by 3-5 days (X = 4.6 days)

2009: Adult control bees outlived adult test bees by an average of 4.2 days



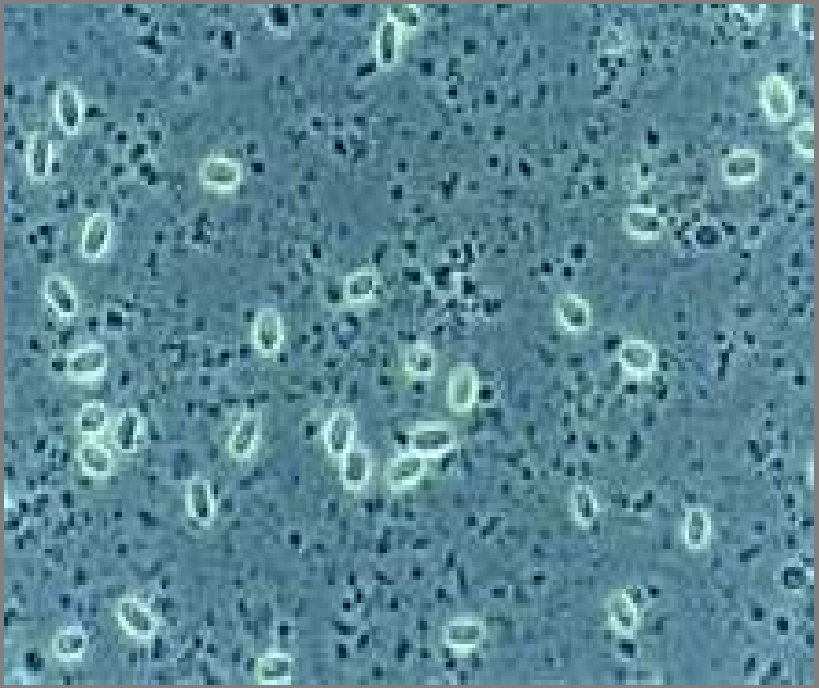
Adult worker emergence

Typical worker emergence occurs at day 20-21



Susceptibility experiments

Examine possible susceptibility to N*osema* infection due to exposure to pesticide residues



Susceptibility experiment

Colony 295 treatment:
formic acid miticide,
no *Nosema* inoculant

(low *Varroa*, normal *Nosema*)

Colony 103 treatment:
no miticide,
Nosema inoculant

(high *Varroa*, high *Nosema*)

-painted control & test bees are put back into each colony

-sample 20 bees from each trt: (week 2, 3, 4 post-release)

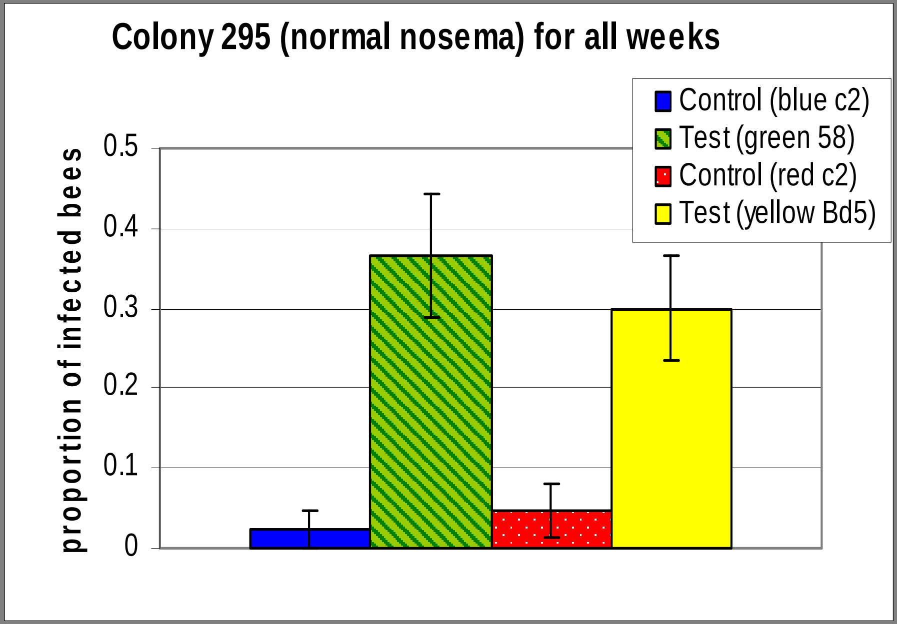
-control & test bees tested for *Nosema* levels

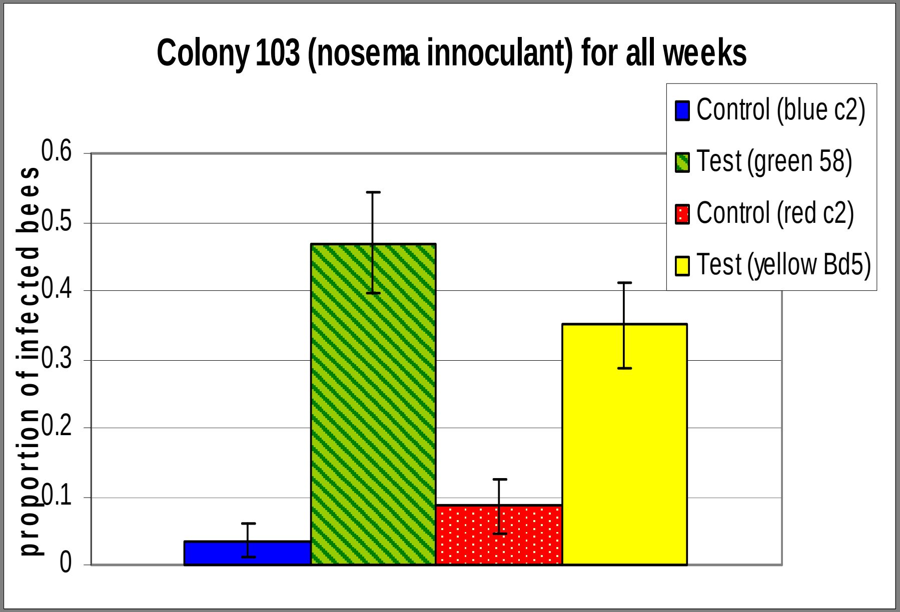
Nosema susceptibility

Samples individually analyzed for *Nosema ceranae* spores

Proportion of “super-infected” bees was higher in both sets of test bees

There’s no difference between colonies! (even with 50 mil spores added each week)

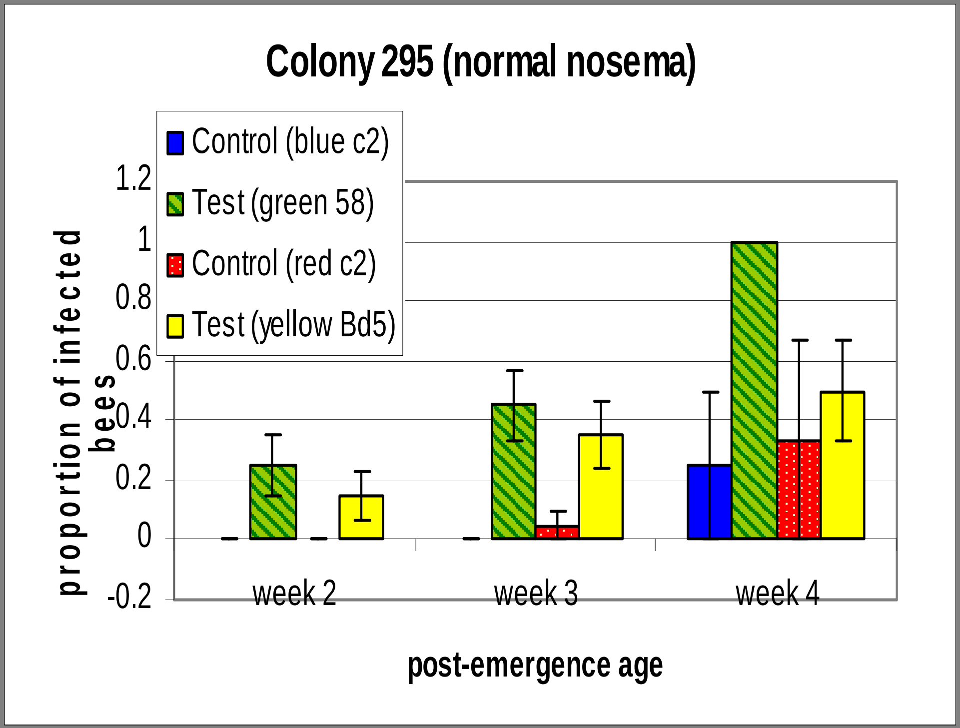


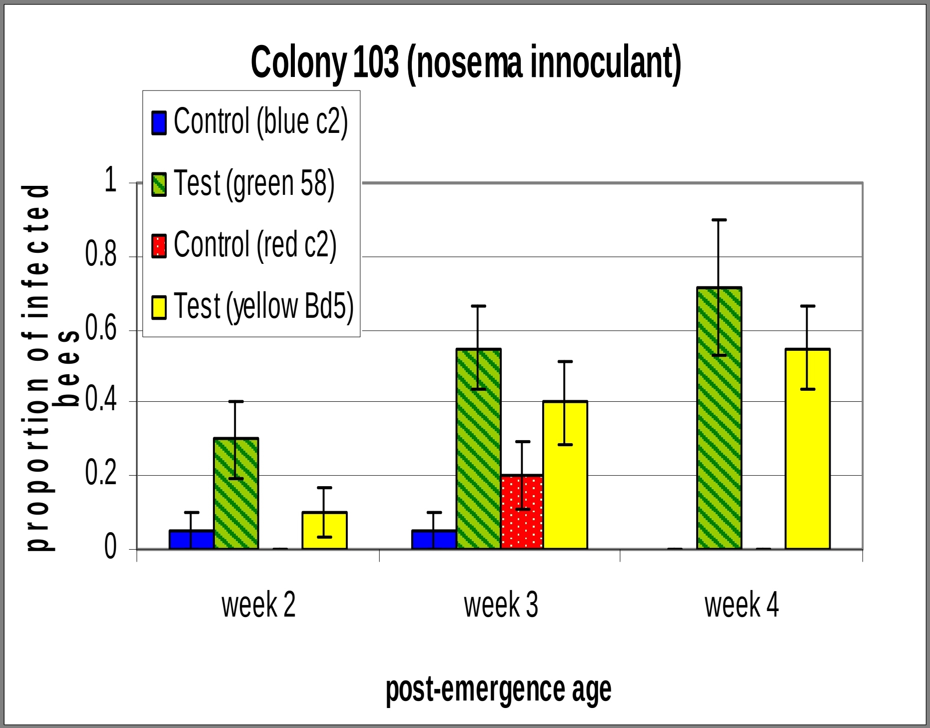


Average # spores

	Colony 295	Colony 103 (added *Nosema*)
Blue (c2)	1,136	62,281
Green (test 58)	51,250	3,164,130
Red (c2)	3,488	170,212
Yellow (test Bd5)	41,000	668,333

Nosema susceptibility





Proportion of infected bees was higher at week 2 in test bees

Proportion of infected bees increased over time in test bees

Large error bars due to small sample numbers at week 4

Summary of findings

Pesticide load survey

77 different pesticides in brood comb (high of 22 in a single comb!)

"Life-table" study

delayed development, lower survivability (egg to adult), delayed emergence

Cage longevity tests

control bees outlived test bees by about 4 days

Behavioral study

Susceptibility experiment

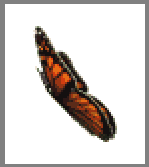
test bees are more susceptible to *Nosema* infection & at a younger age than control bees

Recent Findings on CCD

- 2010 – Researchers find that co-infection of bees with Invertebrate iridescent virus type 6 (IIV-6) and *Nosema ceranae* were found together in all colonies with CCD sampled

(Leal et al. 2010 PLoS ONE 5 (10): e13181.

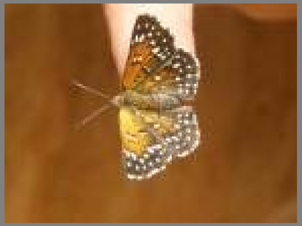
Because exposure to pesticide mixtures have been shown to increase *Nosema* infection rates, pesticides do contribute to CCD



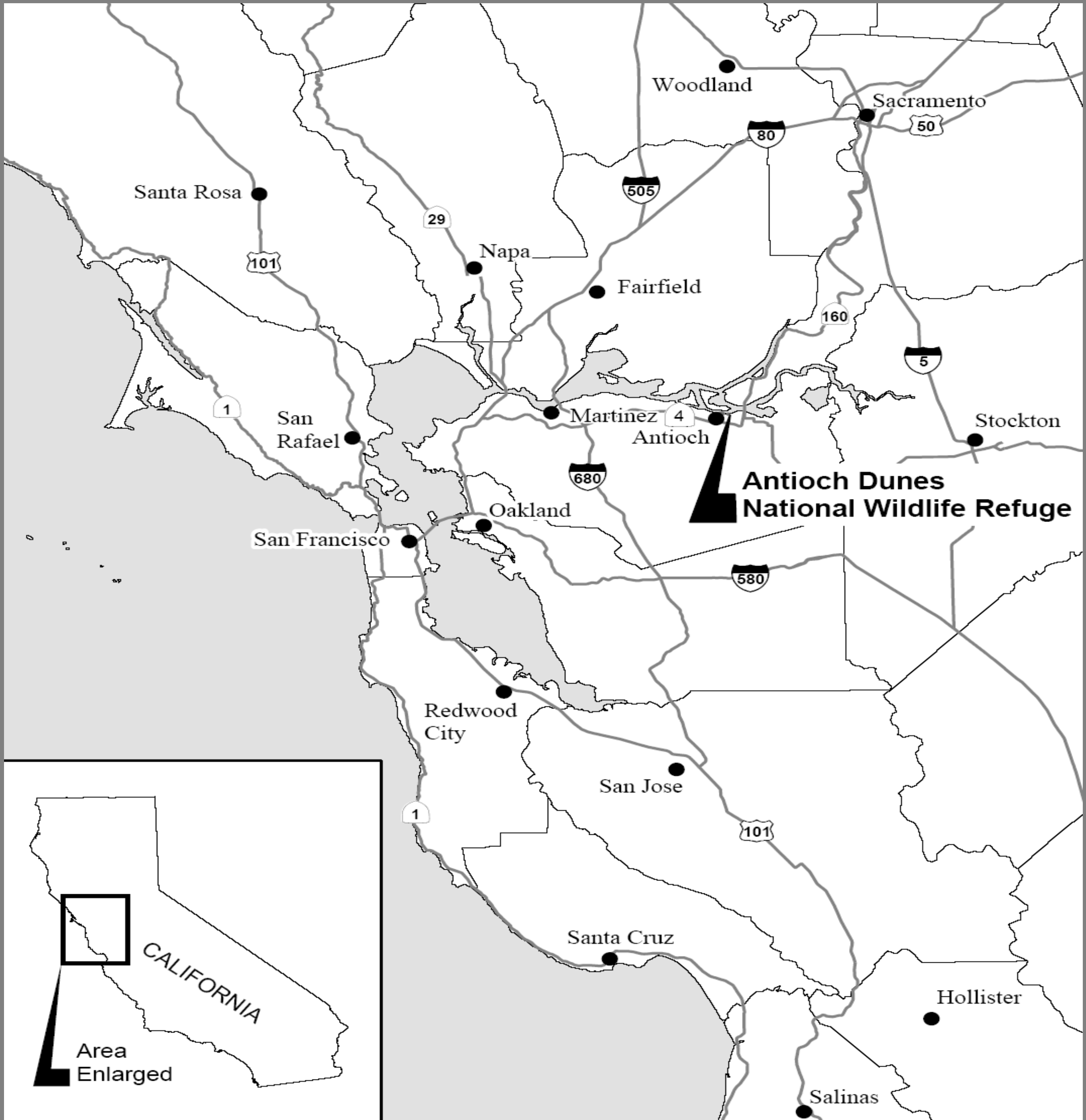
The Effects of Herbicides on Lange's Metalmark Butterfly

John D. Stark[1], Catherine S. Johnson[2], XueDong Chen[1]
[1]Washington State University, Puyallup, WA
[2]U.S. Fish and Wildlife Service, Sacramento, CA

Lange's Metalmark Butterfly



- Federally listed as endangered June 1, 1976
- Antioch Dunes NWR - 1978
- 2007 Biological Opinion – Section 7



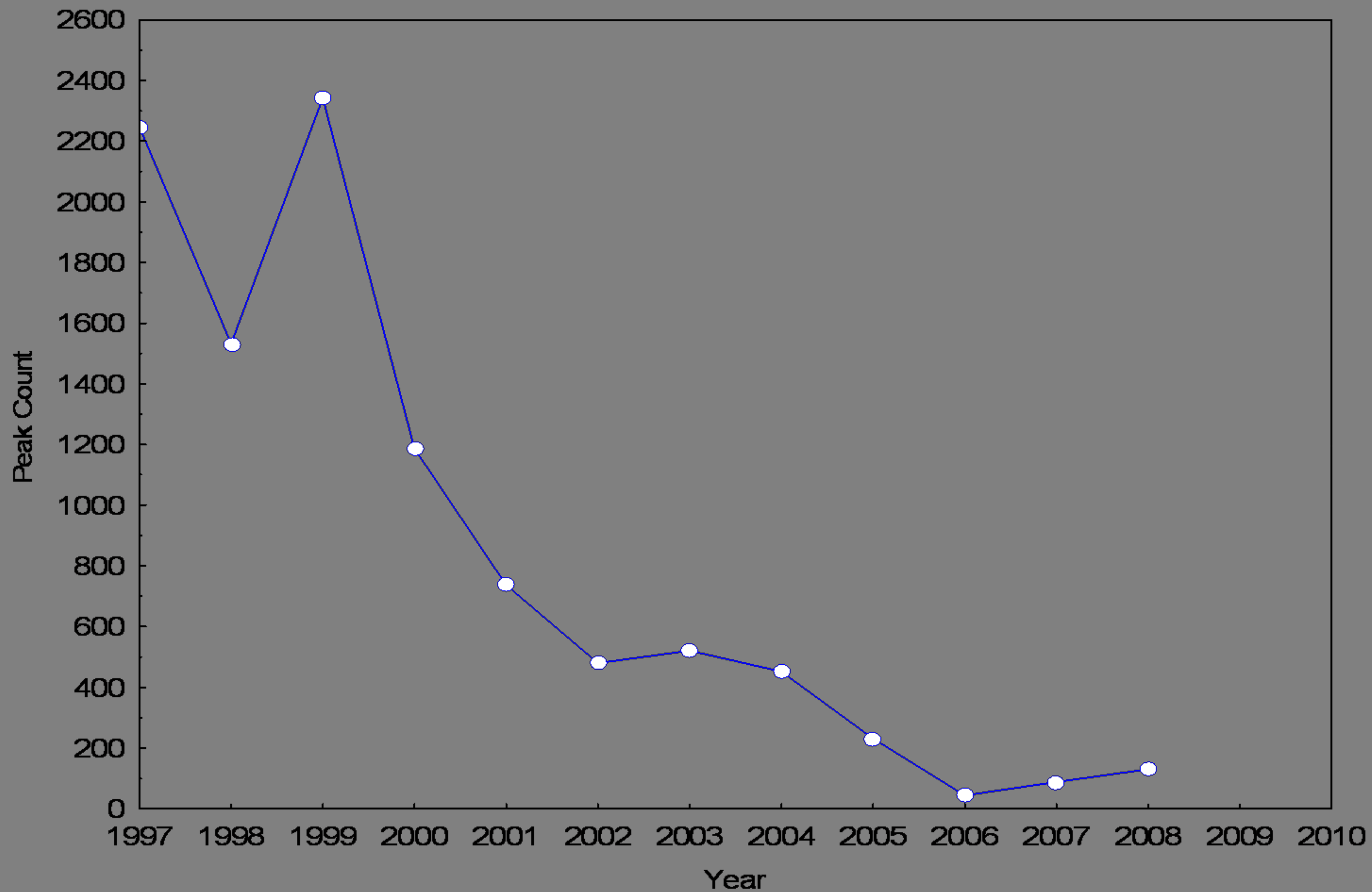
Woodland
Sacramento
80
50
505
Santa Rosa
29
Napa
101
Fairfield
160
5
1
San Rafael
Martinez
4
Antioch
Stockton
680
Antioch Dunes National Wildlife Refuge
Oakland
San Francisco
580
Redwood City
San Jose
1
101
Santa Cruz
Hollister
Salinas
CALIFORNIA
Area Enlarged


Stamm Unit
Sardis Unit
N
Image U.S. Geological Survey
1833 ft
38°00'54.41" N
121°47'24.49" W
Jun 2007
Eye alt 6342 ft
Google

Stamm and Sardis Unit

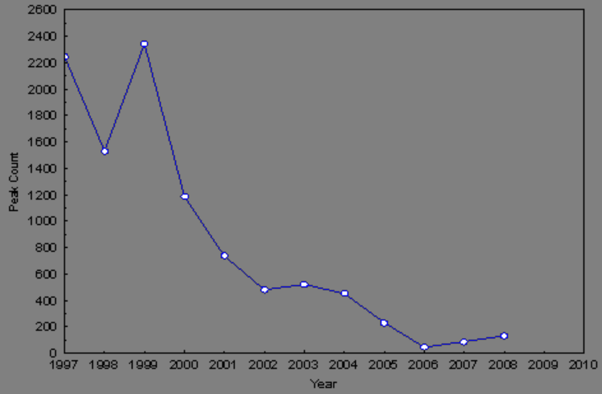
Peak Counts Drastically Go Down

Lange's Metalmark Population Dynamics



What is Being Done

- Aggressive Restoration at Antioch Dunes NWR
- Propagation of Lange's Host Plant
- Captive Rearing of Lange's
- Effects of Herbicide Application



The Problem - Invasive weeds
Specifically Vetch (*Vicia villosa)*

- Alters Micro-Climate
- Encompasses the Buckwheat *nudum)*

Hand Pulling of Vetch Can Damage Buckwheat and Disturb Eggs and Larvae

Herbicide Application

Could the herbicides being applied to remove invasive weeds be having a negative effect on LMB?

Herbicides Used at Antioch Dunes NWR

- Garlon 4 ® - Triclopyr
- Poast ® - Sethoxydim
- Stalker ® - Imazapyr
- Transline ® - Clorpyralid
- Roundup ® - Glyphosate

Objectives

- Determine lethal and sub-lethal effects of three commonly used herbicides at Antioch Dunes NWR on Lange’s metalmark (Garlon 4 –Triclopyr; Poast-Sethoxydim; Stalker –Imazapyr)

- Develop and apply a population life-cycle model to (1) integrate field and laboratory data into population-level projections of the impact of the herbicides on Lange’s metalmark butterfly over developmental time-scales, and (2) provide a comparative assessment of the impact of herbicides relative to other stressors.

We used a surrogate species in the toxicology studies; Behr’s metalmark, (*Apodemia virgulti virgulti).*

Methods

- First instar (larvae) and surrogate buckwheat (Siskiyou wild buckwheat) were exposed at labeled field rates with a Potter Tower

- Daily survival, time to pupation, pupal weight, time to adult emergence, number of emerged adults, adult weight, number of eggs laid per female and a series of other measurements were recorded.

Results for Garlon4

Treatment	Develop. time (d) 1st instar-adult emergence	# pupae produced	# adults produced	Adult longevity (d)	# eggs/female
Control	60.83 ± 2.64	9.50 ± 0.19*	9.50 ± 0.19*	22.83 ± 2.06	29.92 ± 5.81
triclopyr	59.20 ± 2.60	7.25 ± 0.63	7.25 ± 0.63	21.25 ± 2.16	26.11 ± 8.13

A 24% decrease in adults

Results for Poast

Treatment	Develop. time (d) 1st instar-adult emergence	# pupae produced	# adults produced	Adult longevity (d)	# eggs/female
Control	83.51 $\pm$ 4.16	8.87 $\pm$ 1.13*	8.87 $\pm$ 1.13*	28.63 $\pm$ 3.20	25.36 $\pm$ 3.44
sethoxydim	95.52 $\pm$ 3.84	6.50 $\pm$ 1.29	6.50 $\pm$ 1.29	24.95 $\pm$ 4.16	25.91 $\pm$ 2.70

A 27% decrease in adults

Results for Stalker

Treatment	Develop. time (d) 1st instar-adult emergence	# pupae produced	# adults produced	Adult longevity (d)	# eggs/female
Control	114.40 ± 3.05	8.25 ± 0.25*	8.25 ± 0.25*	23.93 ± 2.20	44.00 ± 6.13*
imazapyr	105.10 ± 3.80	5.25 ± 0.63	5.25 ± 0.63	24.12 ± 3.48	20.50 ± 6.87

A 36% decrease in adults

Conclusions

- All three herbicides resulted in a significant decrease in the number of adults that emerged (range 24-36%)