Fungicides and Herbicides Affect Many Aspects of Honey Bee Colony Health

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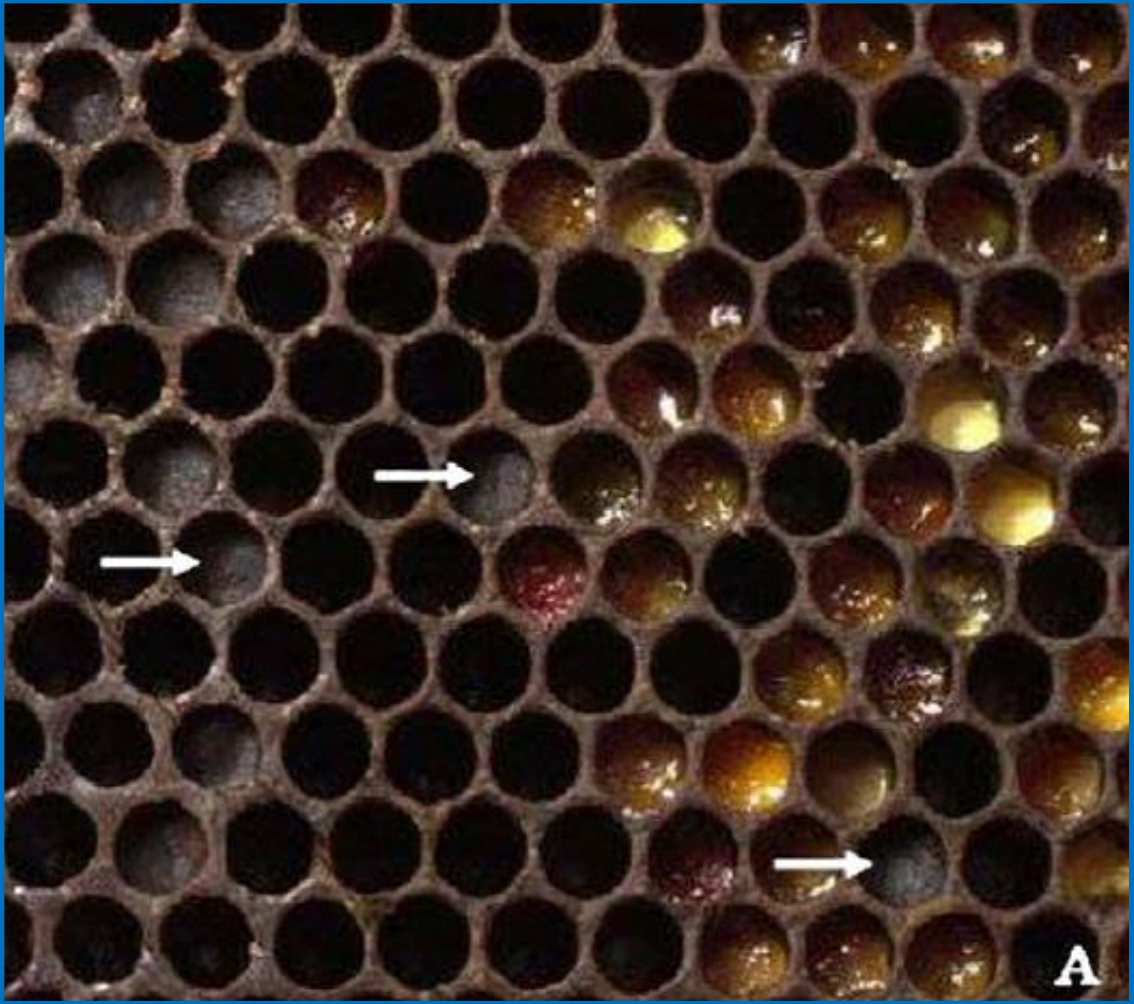
Fungicides Synergize Miticide Toxicity

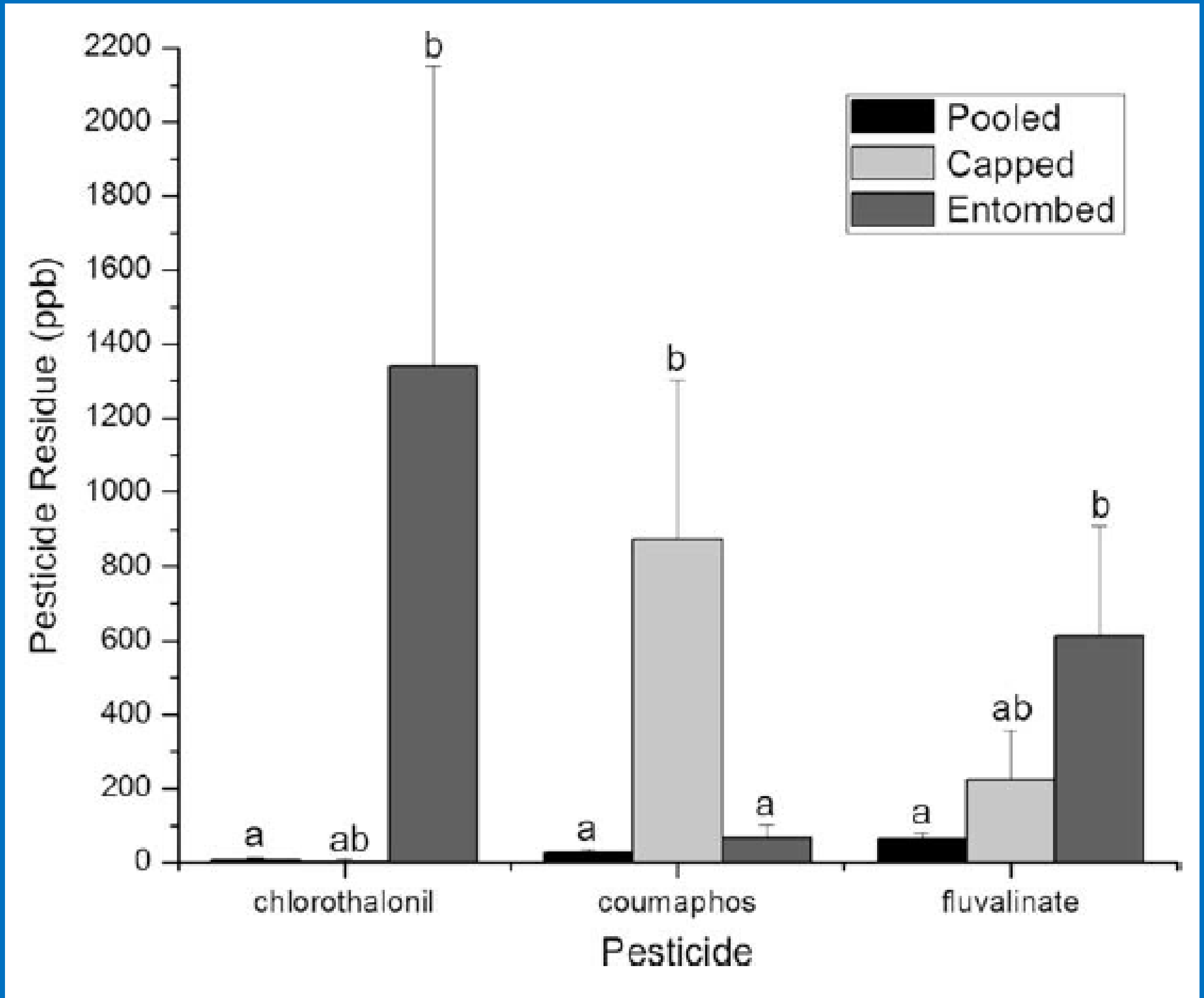
	tau-fluvalinate	coumaphos	fenpyroximate	amitraz	thymol	mode of action
control 1 µl acetone	19.8 16.3-22.4	31.2 22.2-49.6	6.65 4.00-12.0	3.66 2.26-5.56	55.1 42.1-70.0	
fungicides: pyraclostrobin + boscalid 30 µg	5.95[ab] 4.48-8.09	25.9 19.9-34.6	3.16 2.62-3.92	4.04 2.25-10.4	31.9 16.9-44.7	
fungicides: pyraclostrobin 10 µg	4.43[a] 0.67-61.4	-	2.09[a] 0.48-4.24	1.64 0.899-2.51	28.2 4.96-57.9	mitochondrial complex III ubiquinol oxidase inhibitor [32]
fungicides: boscalid 20 µg	11.6 7.43-19.9	22.6 15.3-32.4	5.64 2.89-17.2	4.82 2.83-6.74	47.1 35.4-62.1	mitochondrial complex II succinate dehydrogenase inhibitor [32]
fungicides: chlorothalonil 10 µg	7.24[a] 3.96-12.9	16.6 6.77-85.6	6.41 5.62-7.36	3.34 1.48-8.89	29.8[a] 21.1-39.9	multi-site contact activity [32]
fungicides: prochloraz 10 µg	0.01[a] 0.006-0.017	0.44[a] 0.38-0.50	0.25[a] 0.17-0.34	2.48 1.45-3.74	39.0[b] 33.2-45.1	sterol biosynthesis (P450) inhibitor [32]

LD_{50} fold-change relative to control							
<1		1	2	5	20	50	100

Johnson 2013 PLoS One 8:e54902

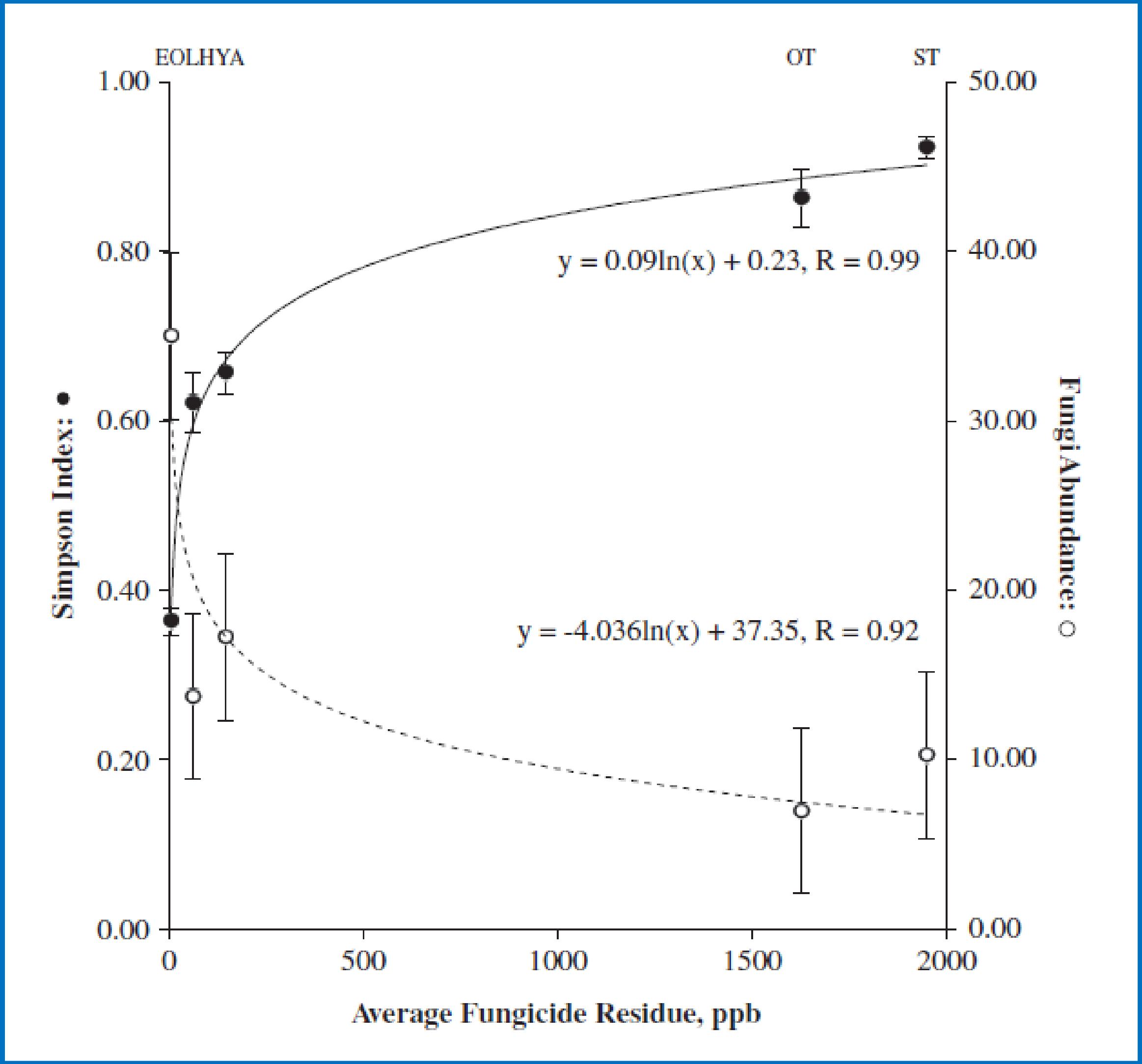
High Levels of Chlorothalonil in Entombed Pollen





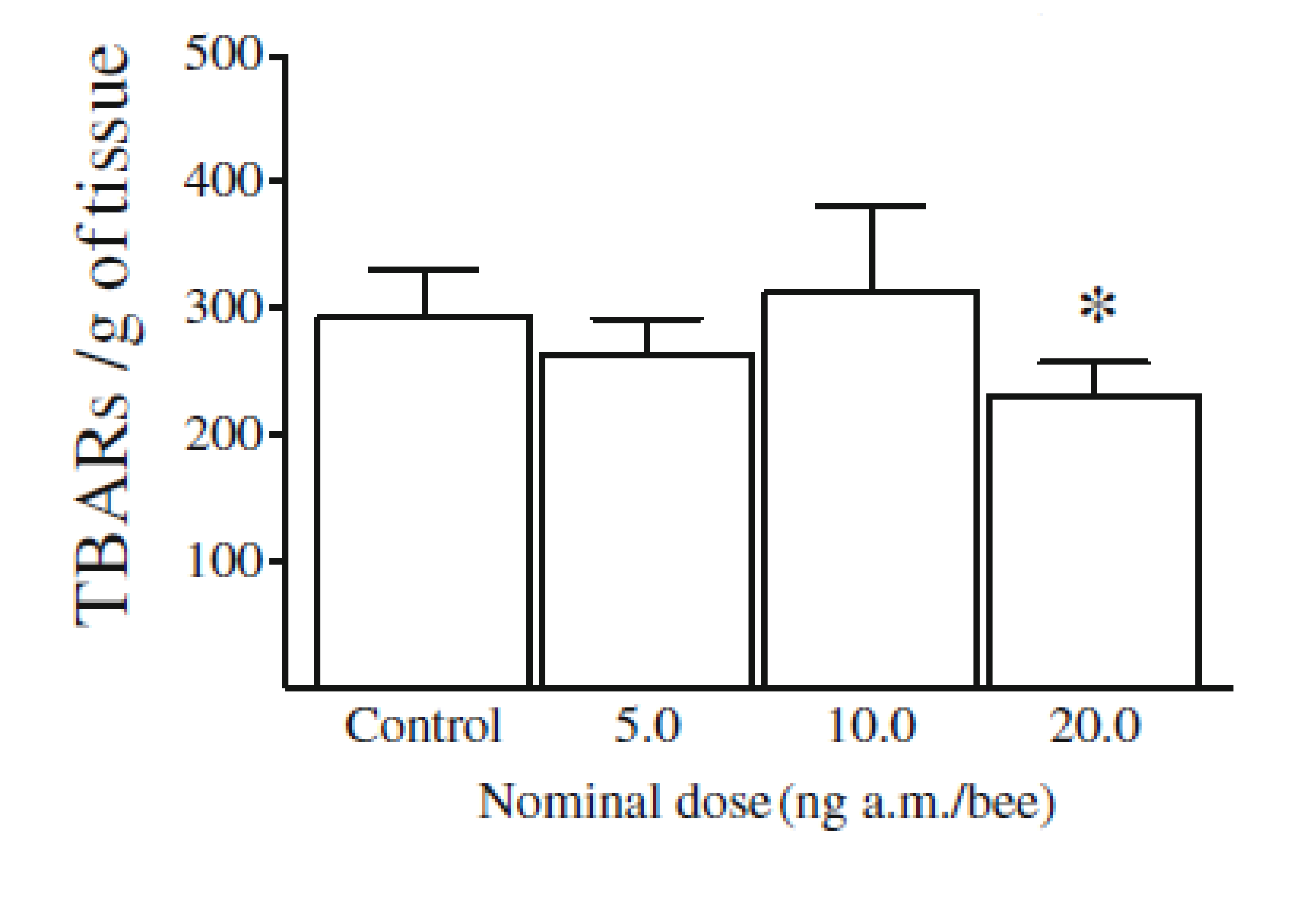
vanEngelsdorp 2009 J Inv Path 101:147

Fungicides Affect Microbiota of Bee Bread



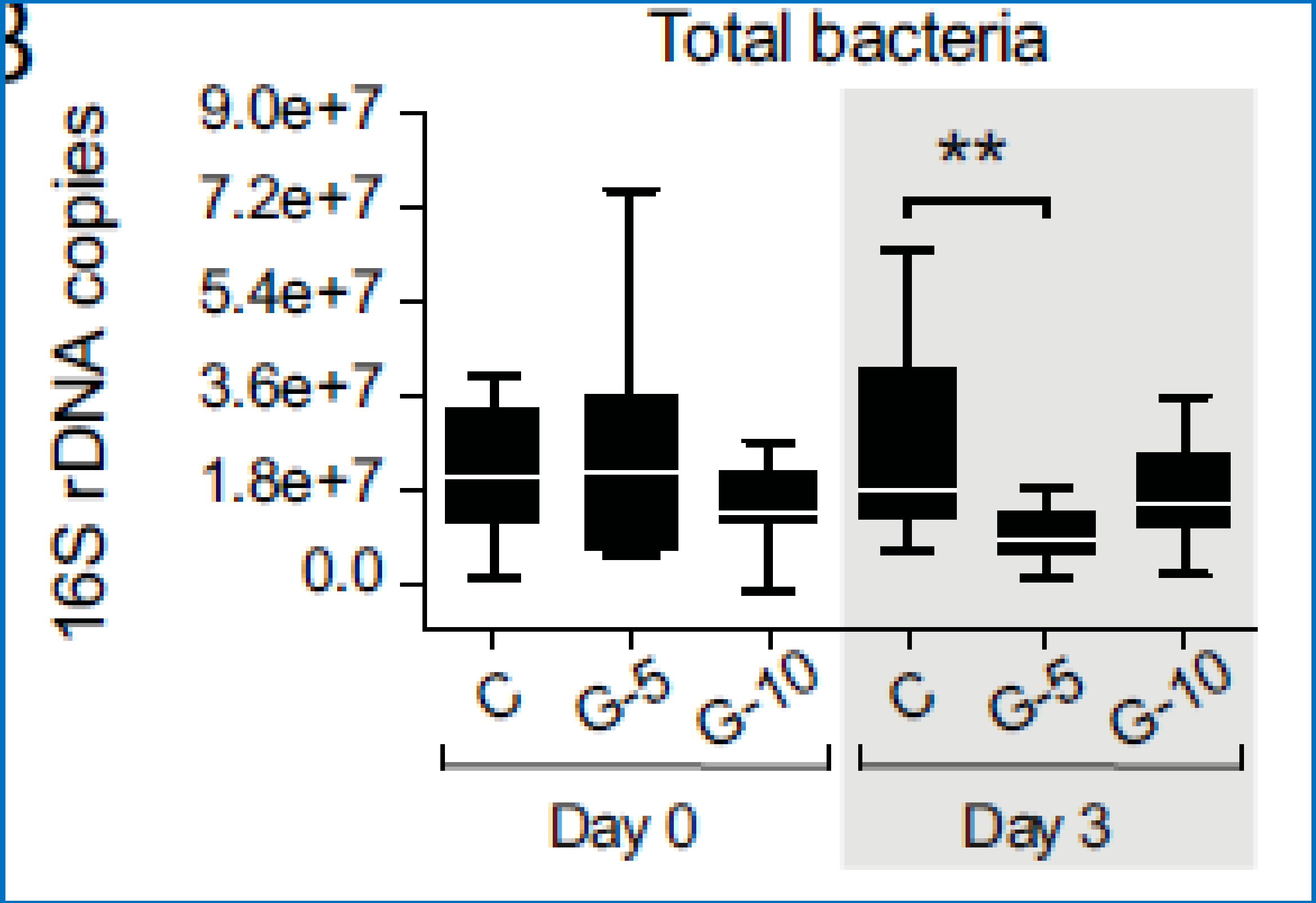
Yoder 2013 J Tox Env Health 76:587

Metolachlor Alters Lipid Peroxidation



Helmer 2015 Env Sci Pol Res 22:8010

“Glyphosate Perturbs the Gut Microbiota of Honey Bees”



Motta 2018 PNAS 115:10305

“Minimizing the Impact of Varroa Mites and Mite-Borne Pathogens on Managed Honey Bees”

1. Determine if Pol-Line can mitigate losses from *Varroa* and associated viruses in a commercial operation

2. <u>Link risk factors to real-world outcomes</u>

3. Effects of migration route and overwintering location

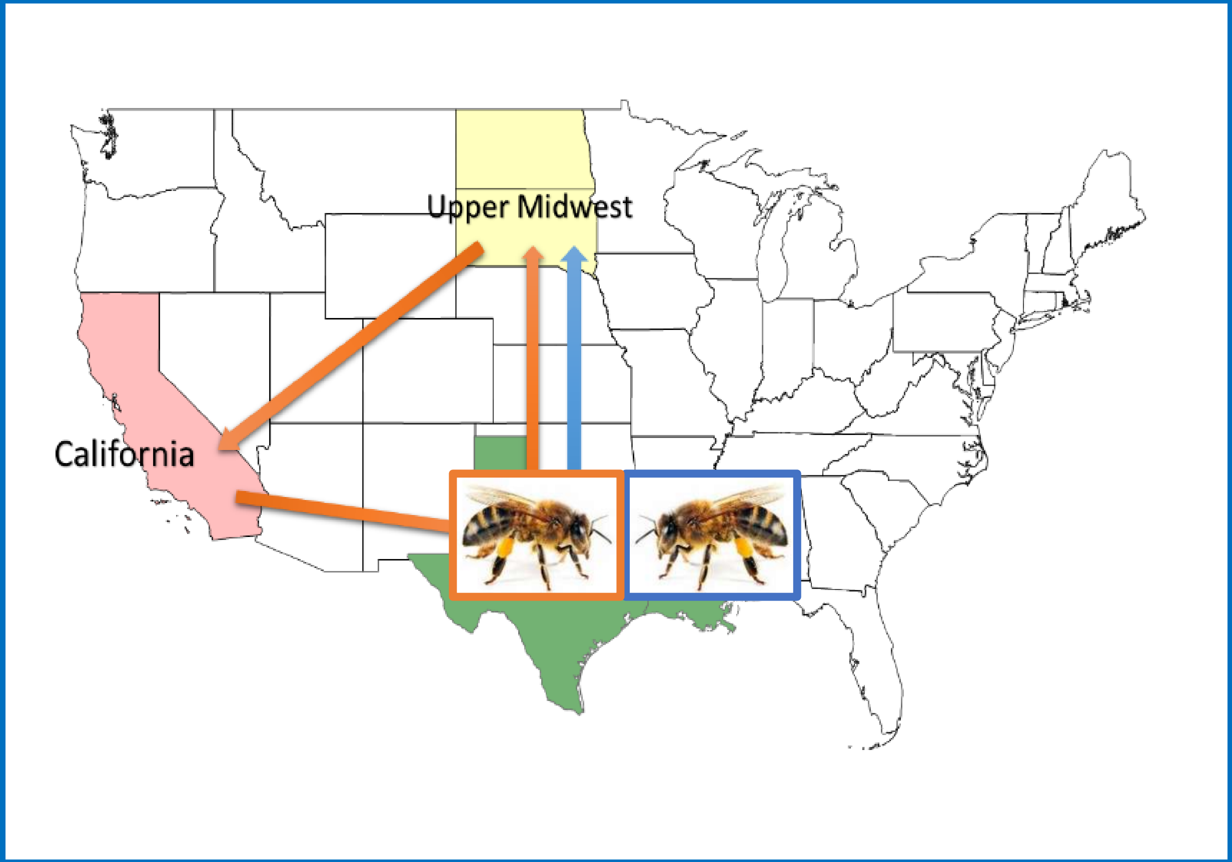
4. Develop epidemiological models

2017-18 Migratory Project Colony Numbers

171 Pol-Line and 191 Commercial

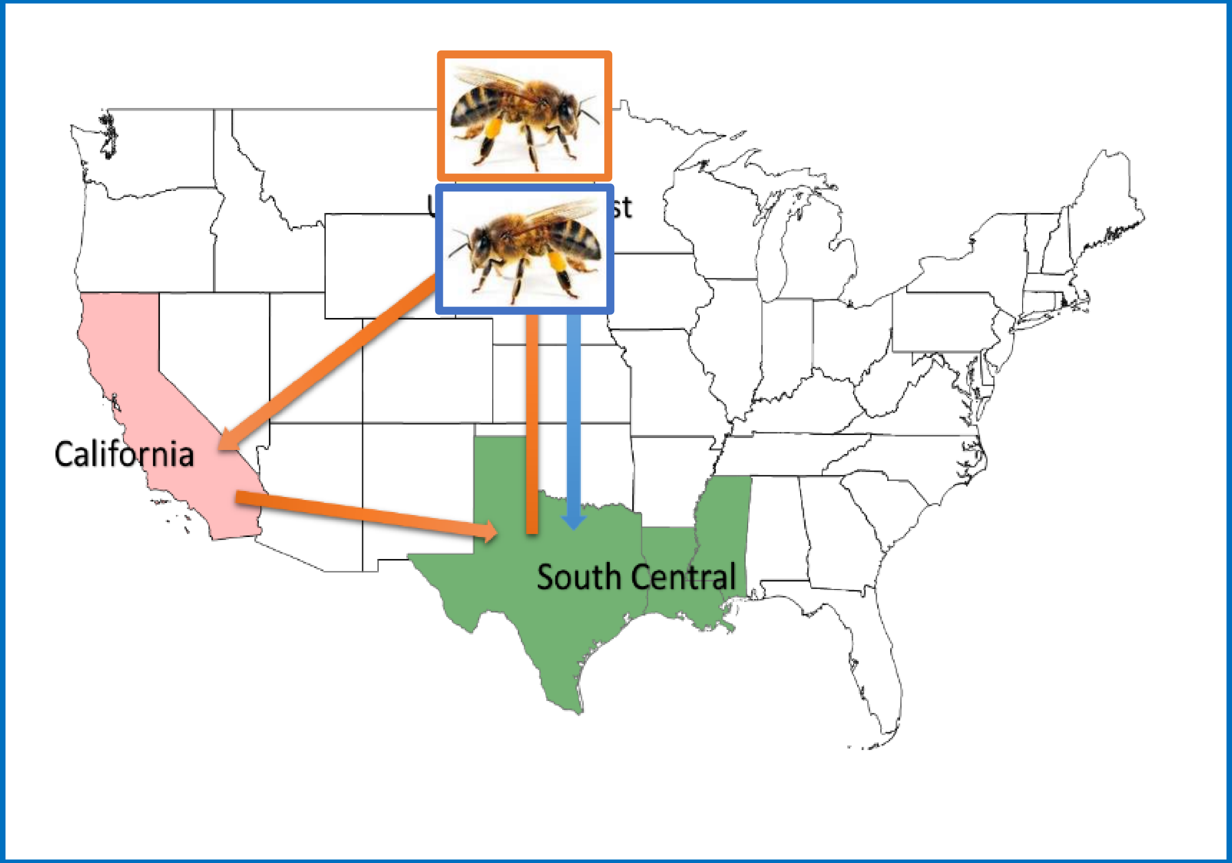
Colony Sampling Intervals

1. Colony Establishment, LA/MS, April 2017



Colony Sampling Intervals

1. Colony Establishment, LA/MS, April 2017
2. Post-Migration, ND/SD, June 2017



Colony Sampling Intervals

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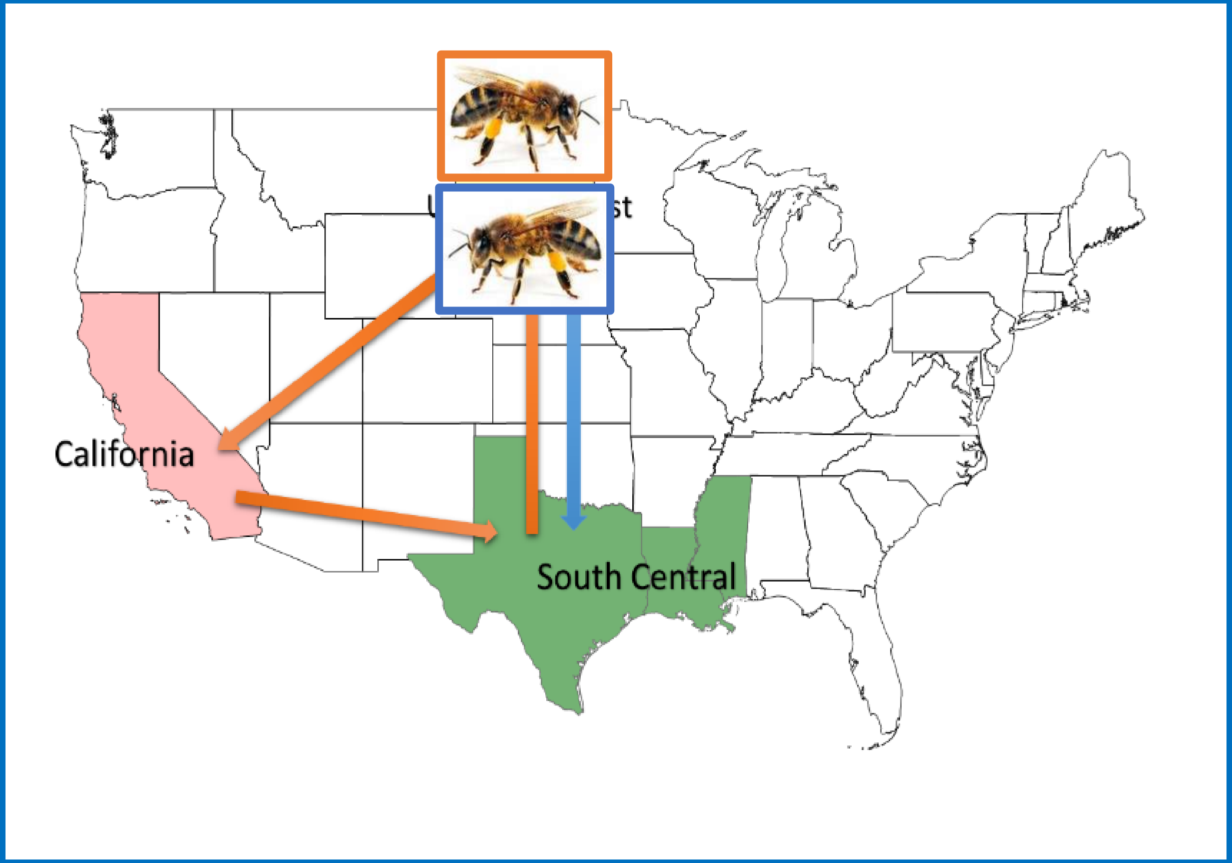

Colony Sampling Intervals

1. Colony Establishment, LA/MS, April 2017
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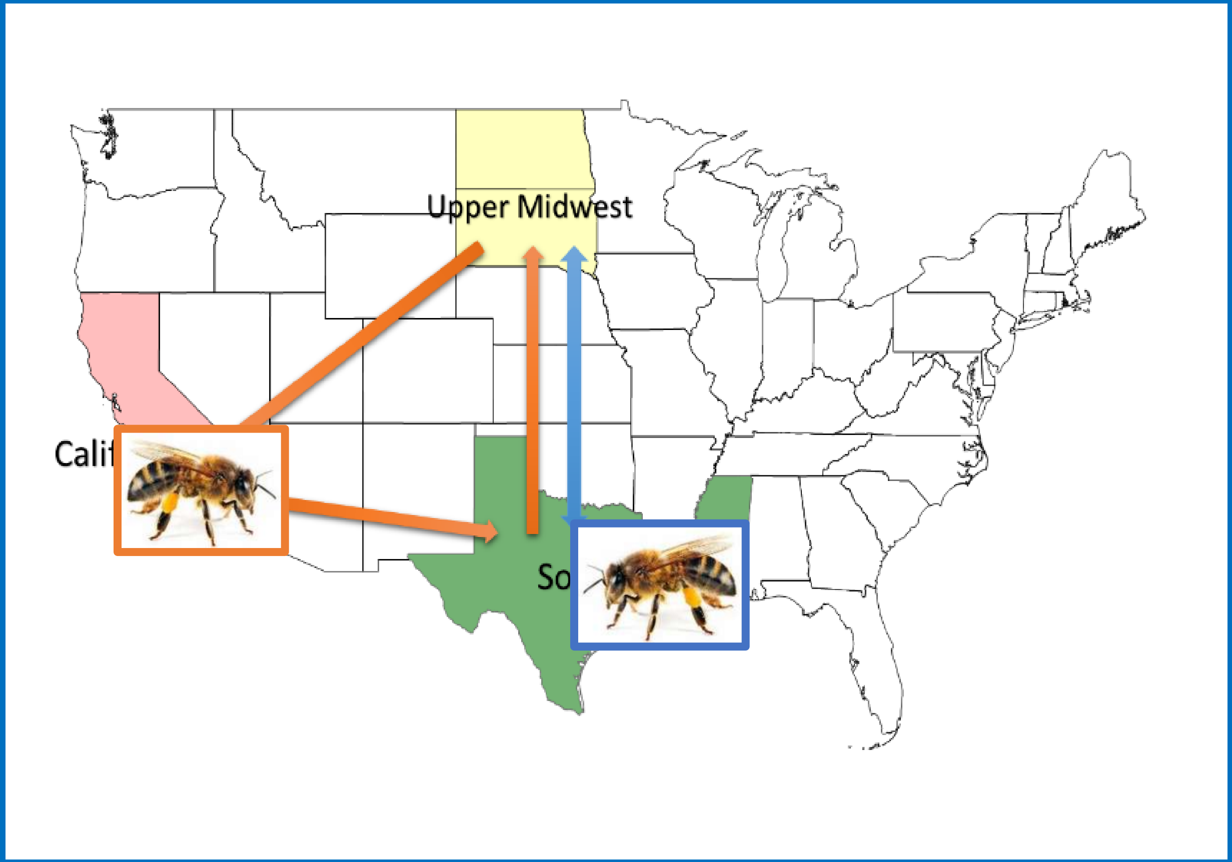
4. Overwinter, CA or MS, December 2017



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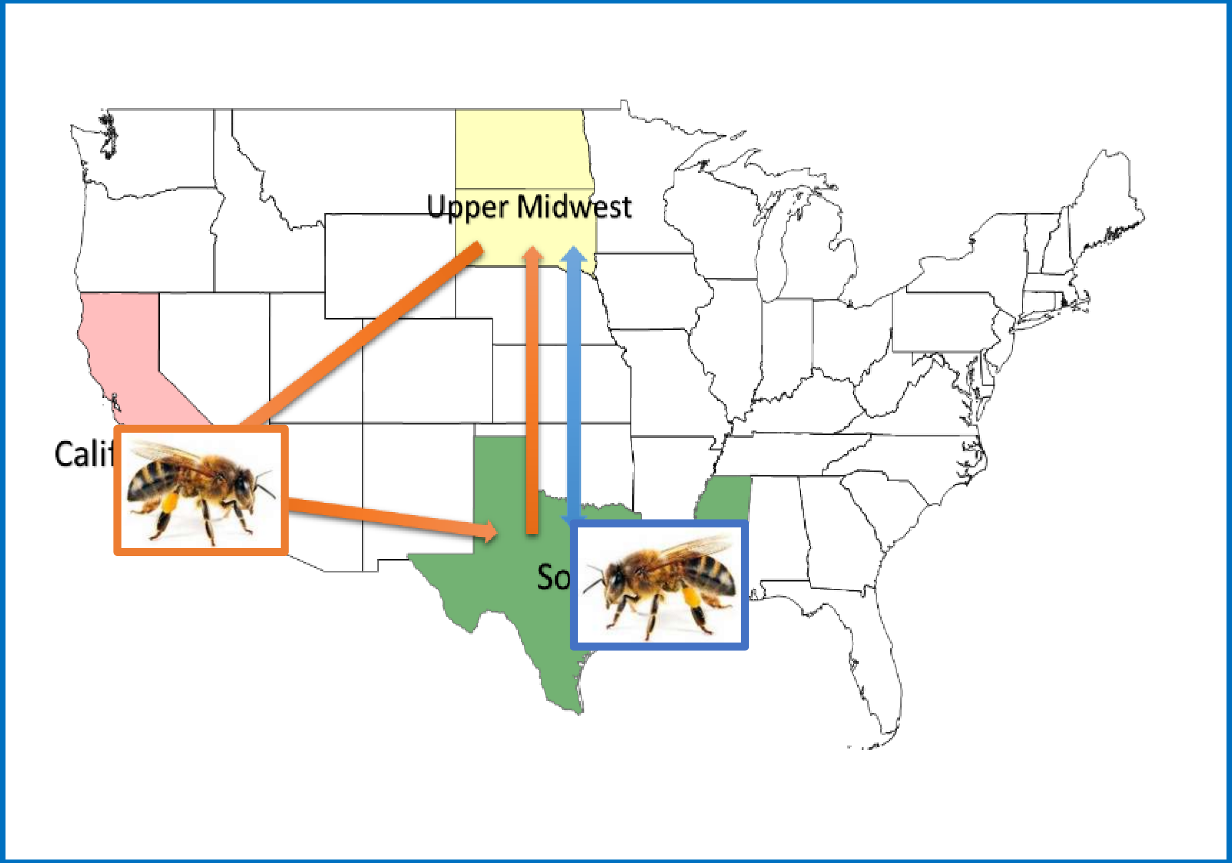
3. Honey Harvest, ND/SD, September 2017
4. Overwinter, CA or MS, December 2017
5. Almonds, CA, February 2018
   No Almonds, MS, February 2018



Colony Sampling Intervals

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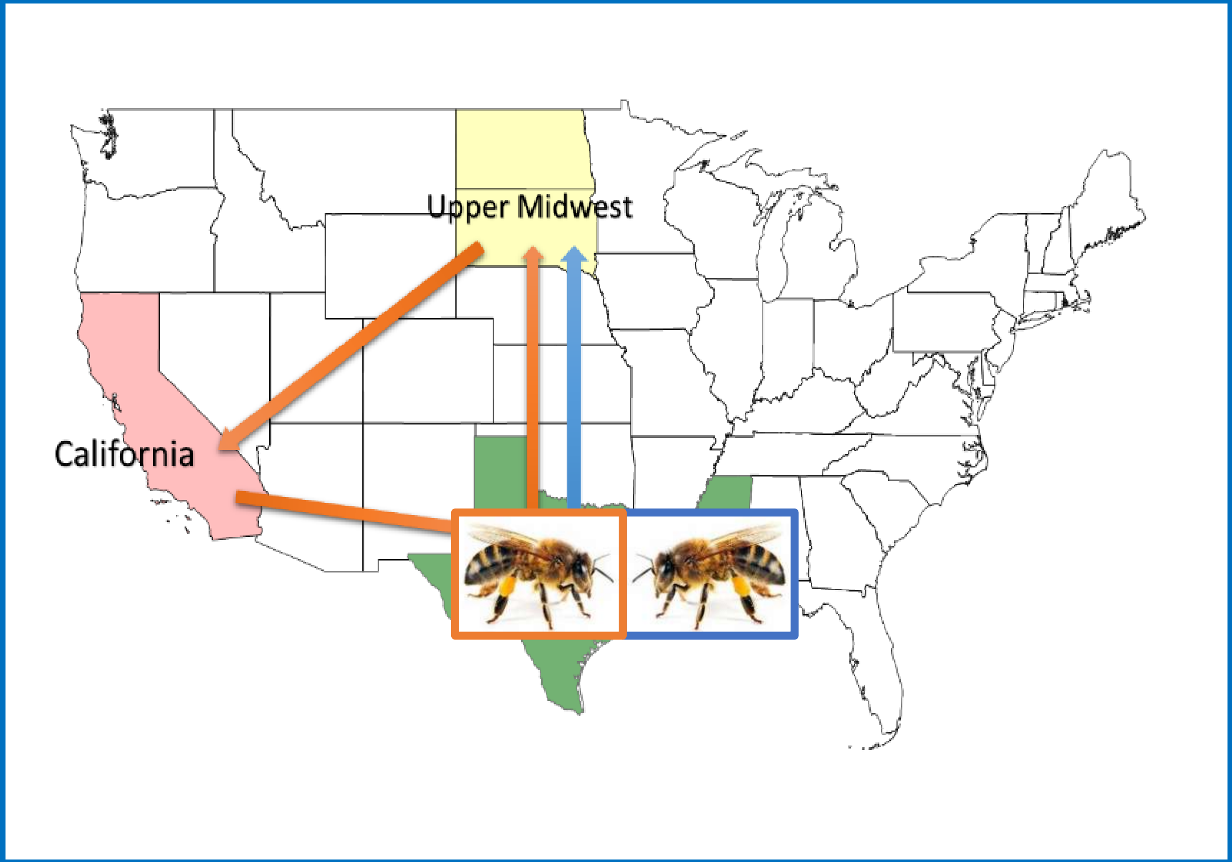
   No Almonds, MS, February 2018
6. Spring Build Up, MS, April 2018



Colony Measurements

<u>Pesticide exposure</u>

Queen status

Bee population

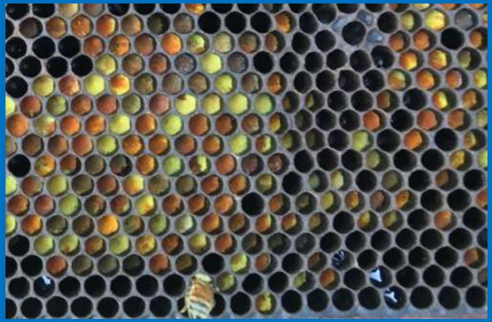
Honey production

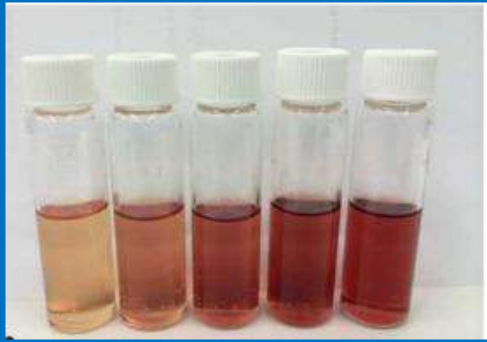
Brood area

Pollen and nectar stores

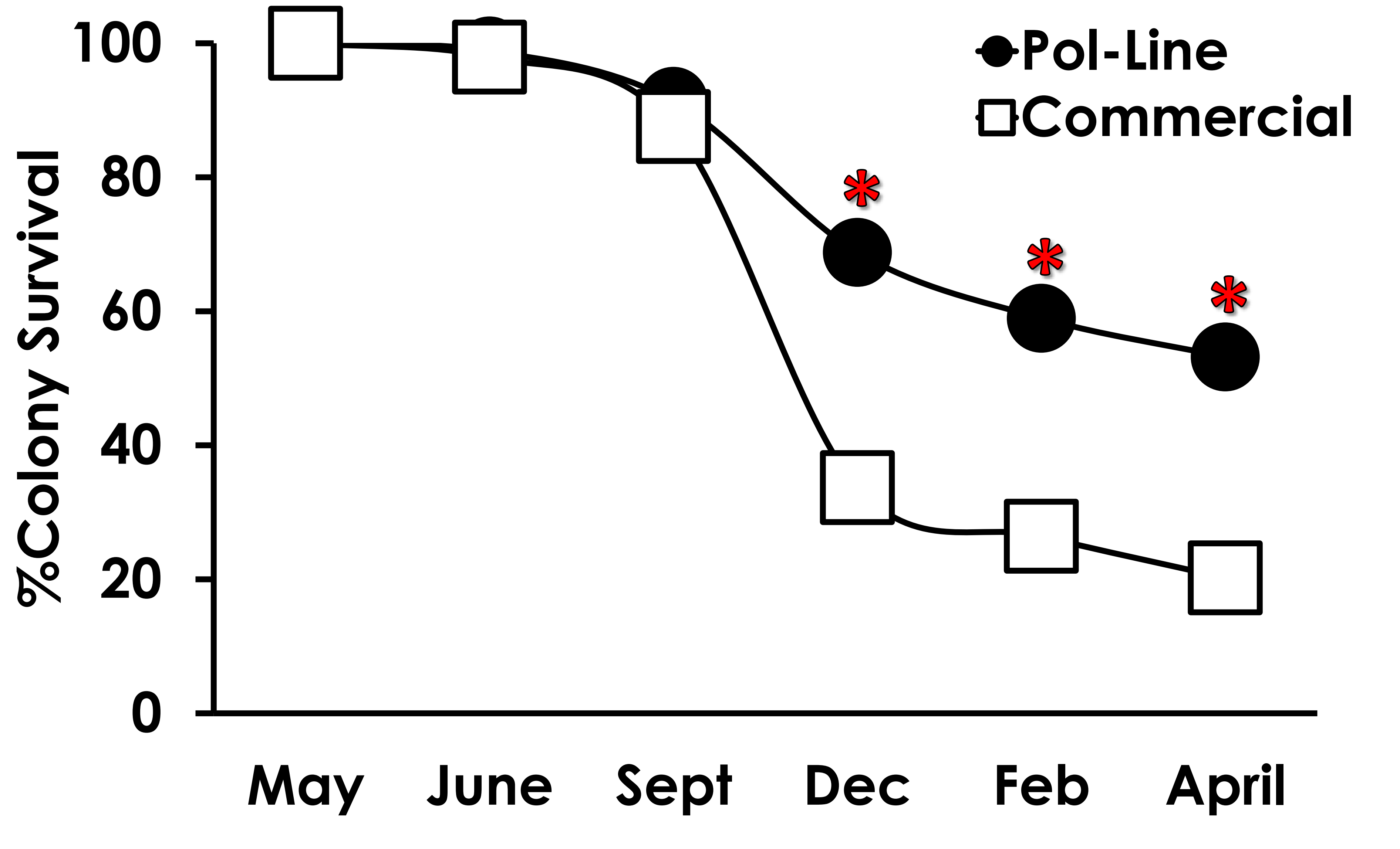
Varroa infestation level

Virus levels

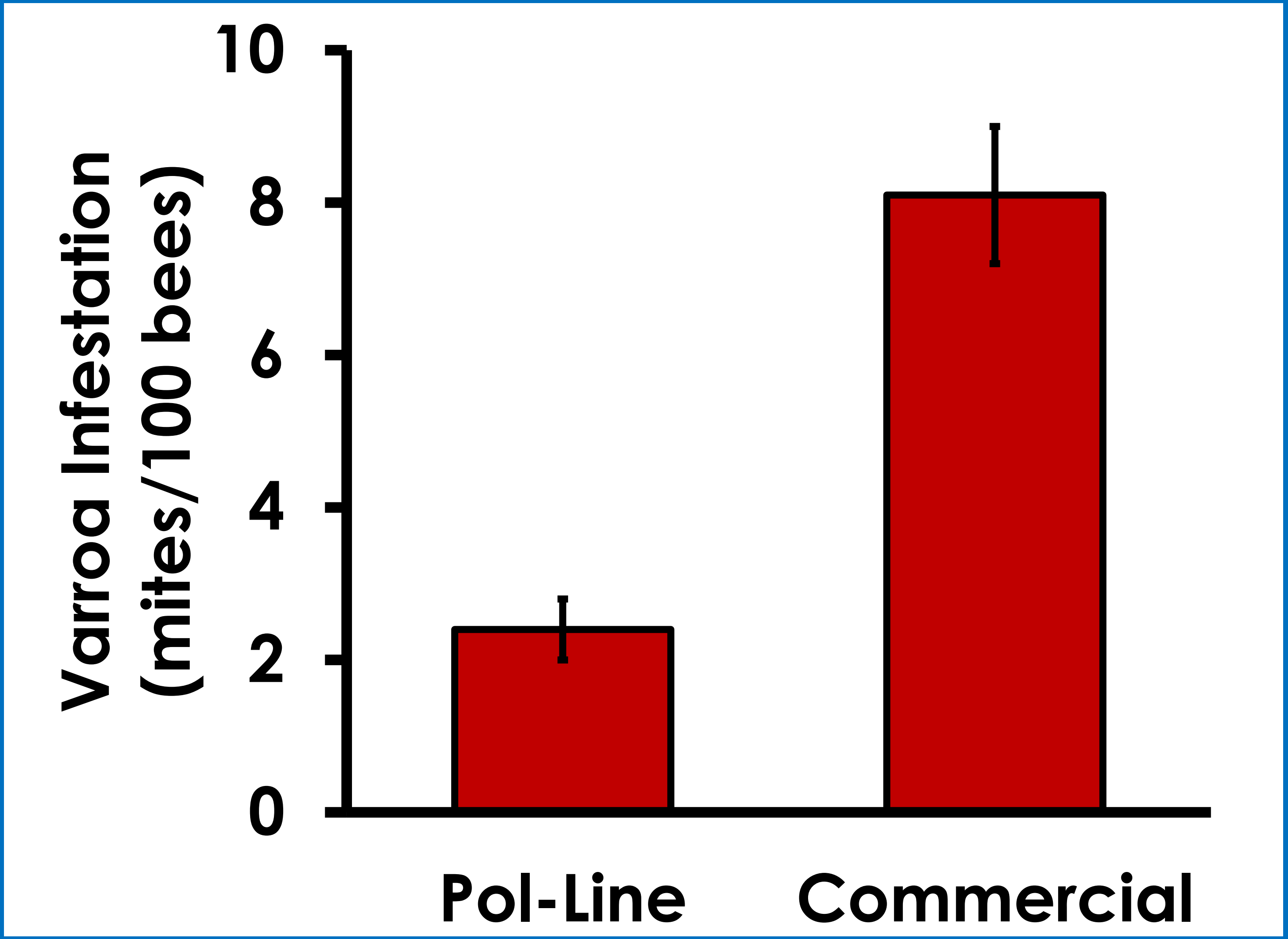




Pol-Line Colonies Have Higher Survival Rate



Pol-Line Has Fewer *Varroa* Mites After Honey Harvest in September



Post-Hoc Pesticide Residue Tests

Survived vs Died

Samples Selected by Standardized Varroa Infestation Rate

September Pollen and Nectar Samples Analyzed at NSL Gastonia

Results

Only Amitraz (DMPF) Found in Nectar Samples

No Neonicotinoids Were Detected

Pesticides Detected in >10% of Samples

Pesticide	Class	% Detections	Mean PPB
2,4 Dimethylphenyl formamide (DMPF)	Miticide	94.5	244.2
Chlorpyrifos	Insecticide	70.3	7.2
Azoxystrobin	Fungicide	36.3	3.3
Chlorothalonil	Fungicide	27.5	610.7
Coumaphos	Miticide	23.1	26.6
Thymol	Miticide	19.8	165.1
Boscalid	Fungicide	18.7	10.1
Piperonyl butoxide	Insecticide	15.4	39.6
Trifloxystrobin	Fungicide	14.3	0.7
Acetochlor	Herbicide	13.2	131.1
Coumaphos oxon	Miticide	12.1	2.0
Metolachlor	Herbicide	12.1	42.4

Diverse and Consistent Pesticide Exposure

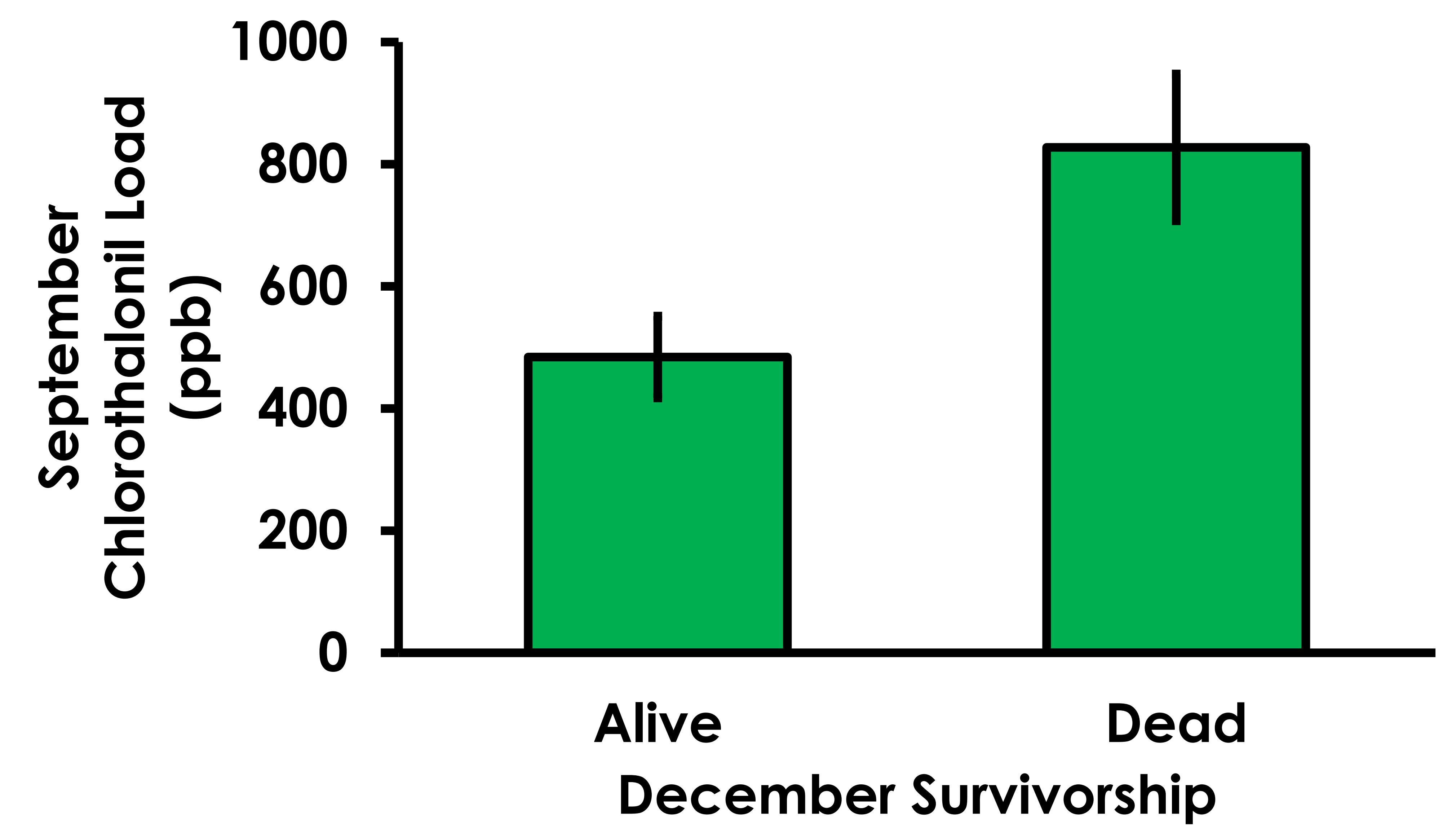
Class	N-Types	N	%
Overall	36	91	100.0
Miticides	5	89	97.8
Insecticides	8	69	75.8
Fungicides	15	60	65.9
Herbicides	8	24	26.4

Apiary	Shannon	Evenness
Bin5	2.37	0.86
F3	2.48	0.88
MD12	2.65	0.85
Yard44	2.18	0.77

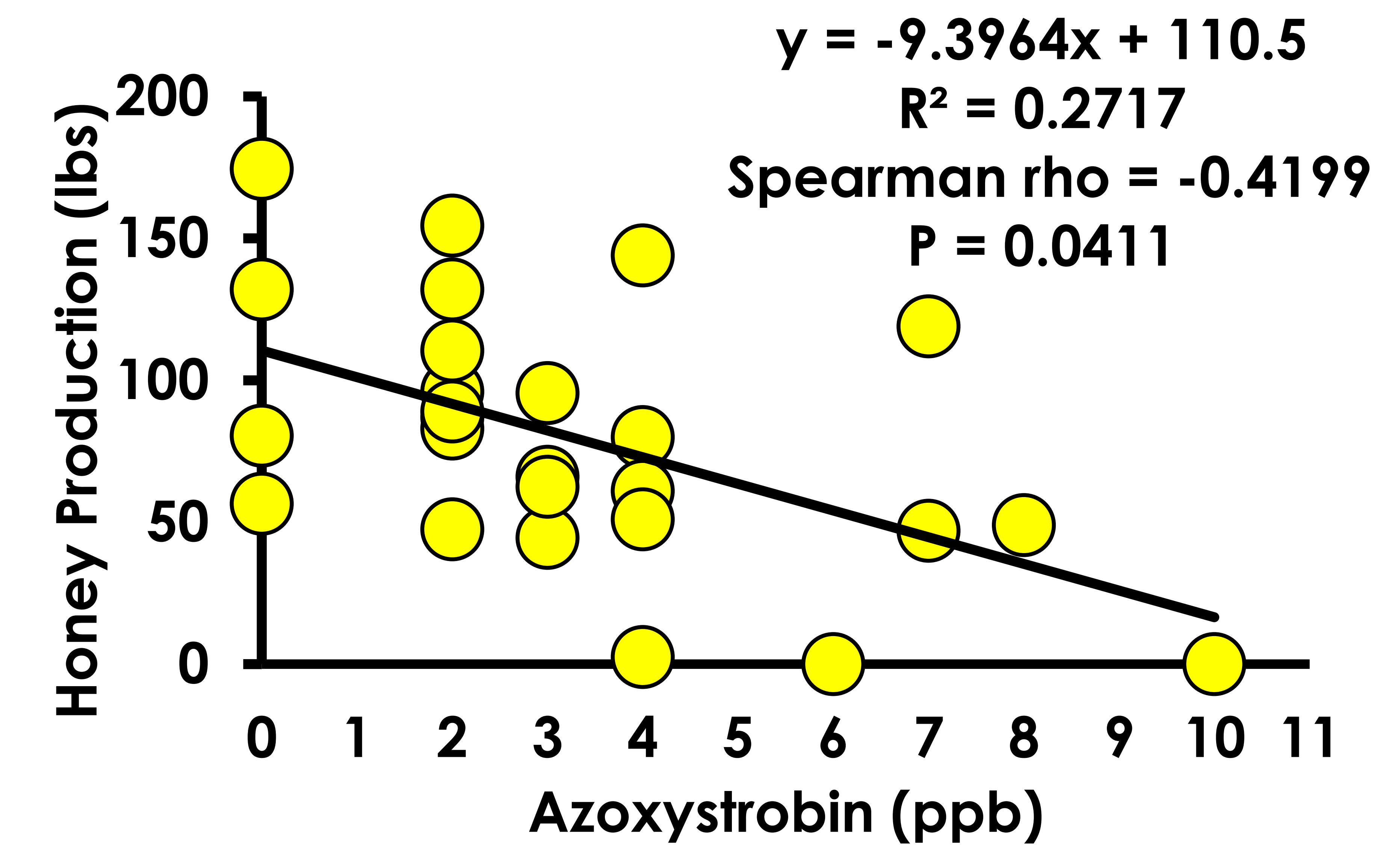
Exposures Associated with Colony Losses

Mortality	Total			Miticide			Insecticide			Fungicide			Herbicide		
Month	Count	Load	HQ	Count	Load	HQ	Count	Load	HQ	Count	Load	HQ	Count	Load	HQ
December			X								X	X			
February		X	X							X	X	X	X	X	X
April		X									X	X	X		

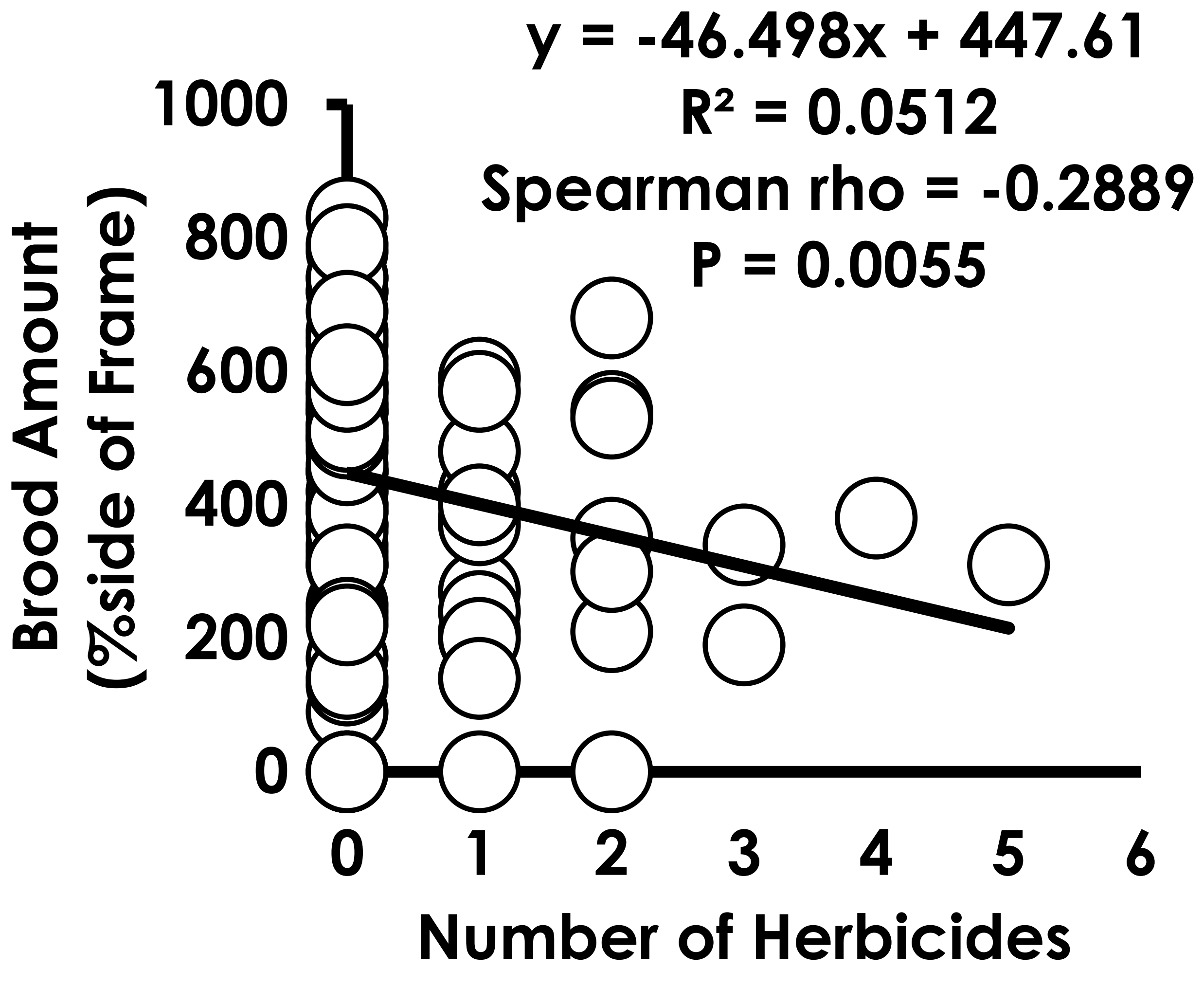
High Chlorothalonil Associated with December Colony Losses

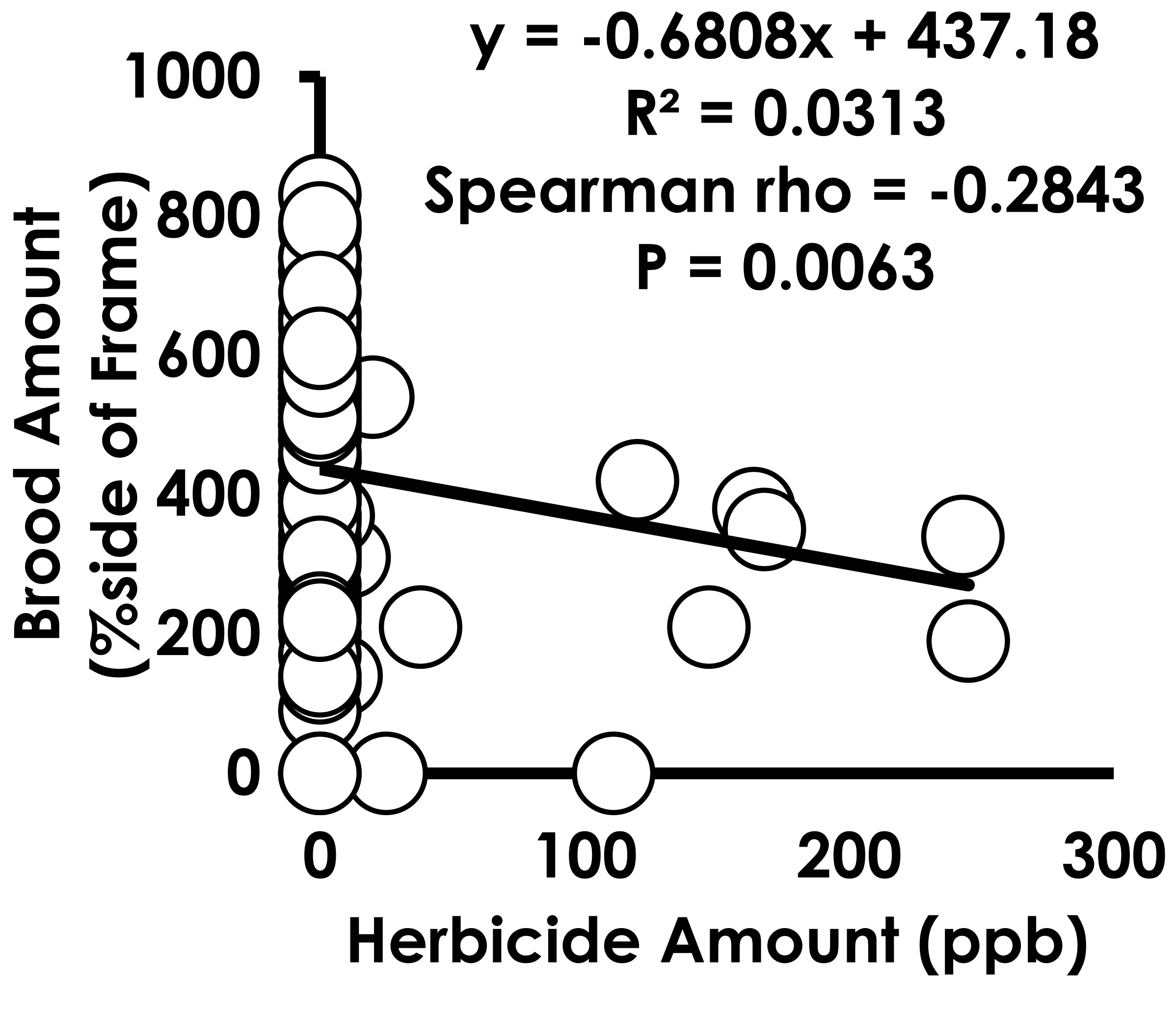


Reduced Honey Production with Increasing Azoxystrobin

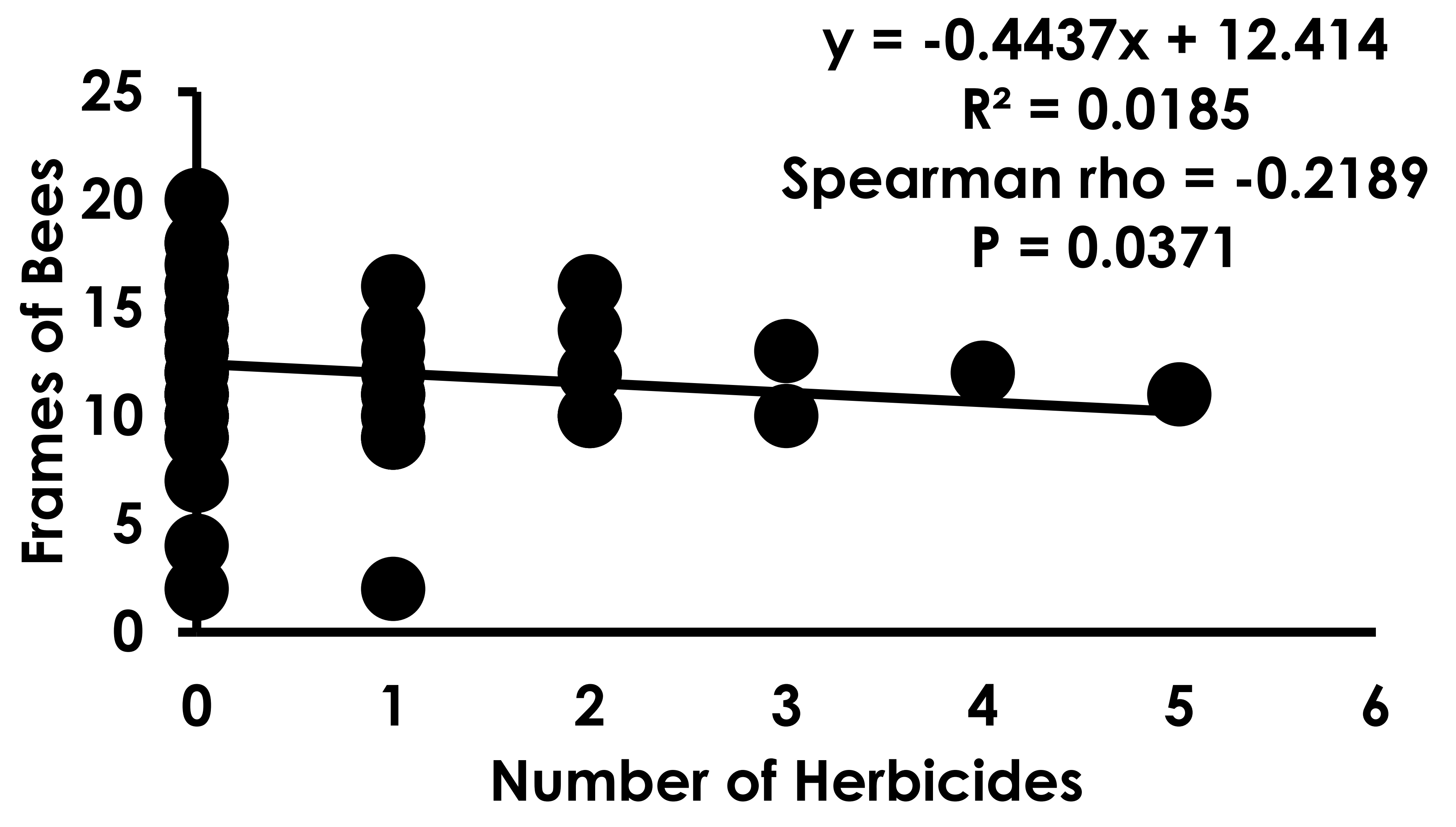


Less Brood Area with Increasing Number and Amount of Herbicides

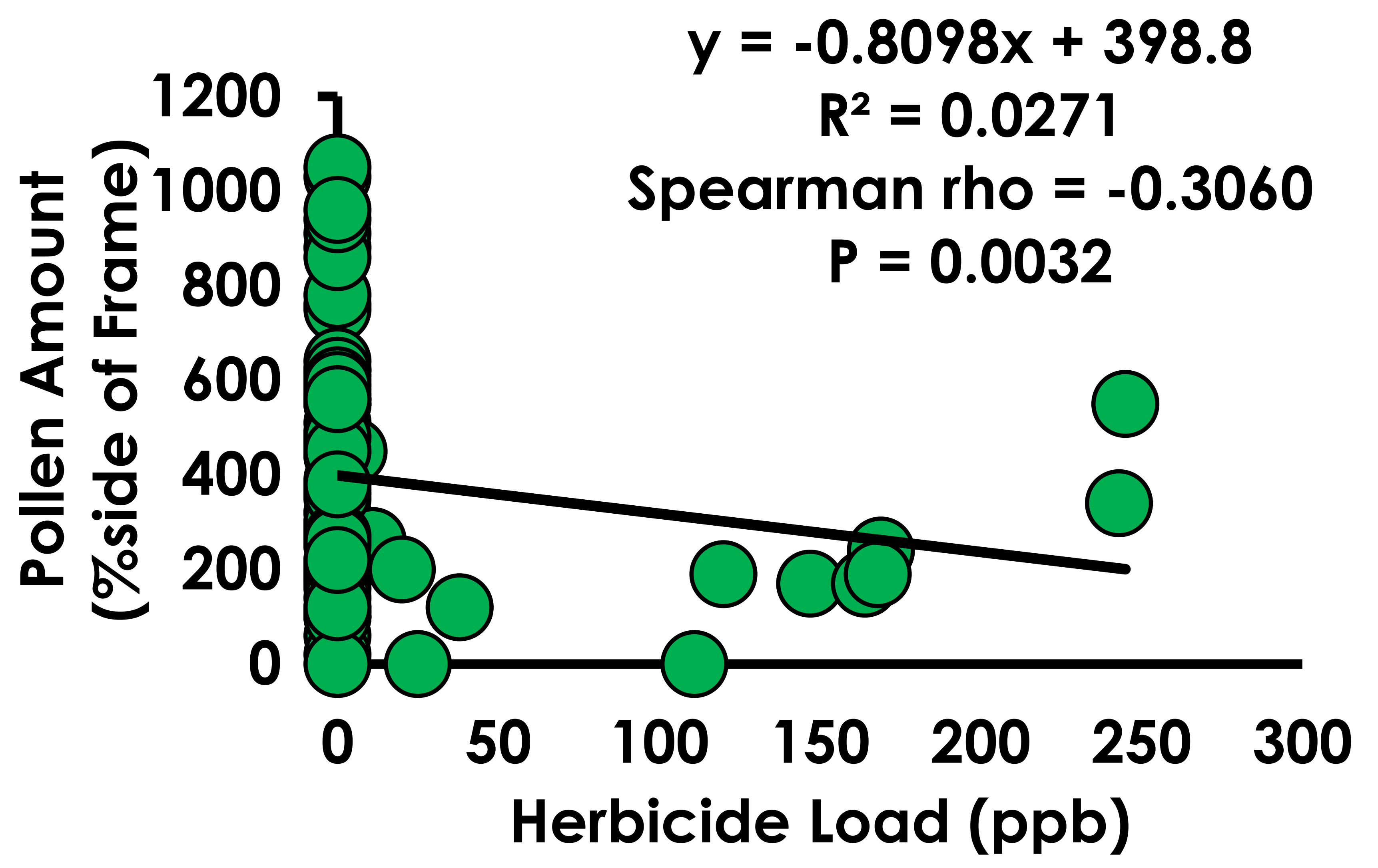




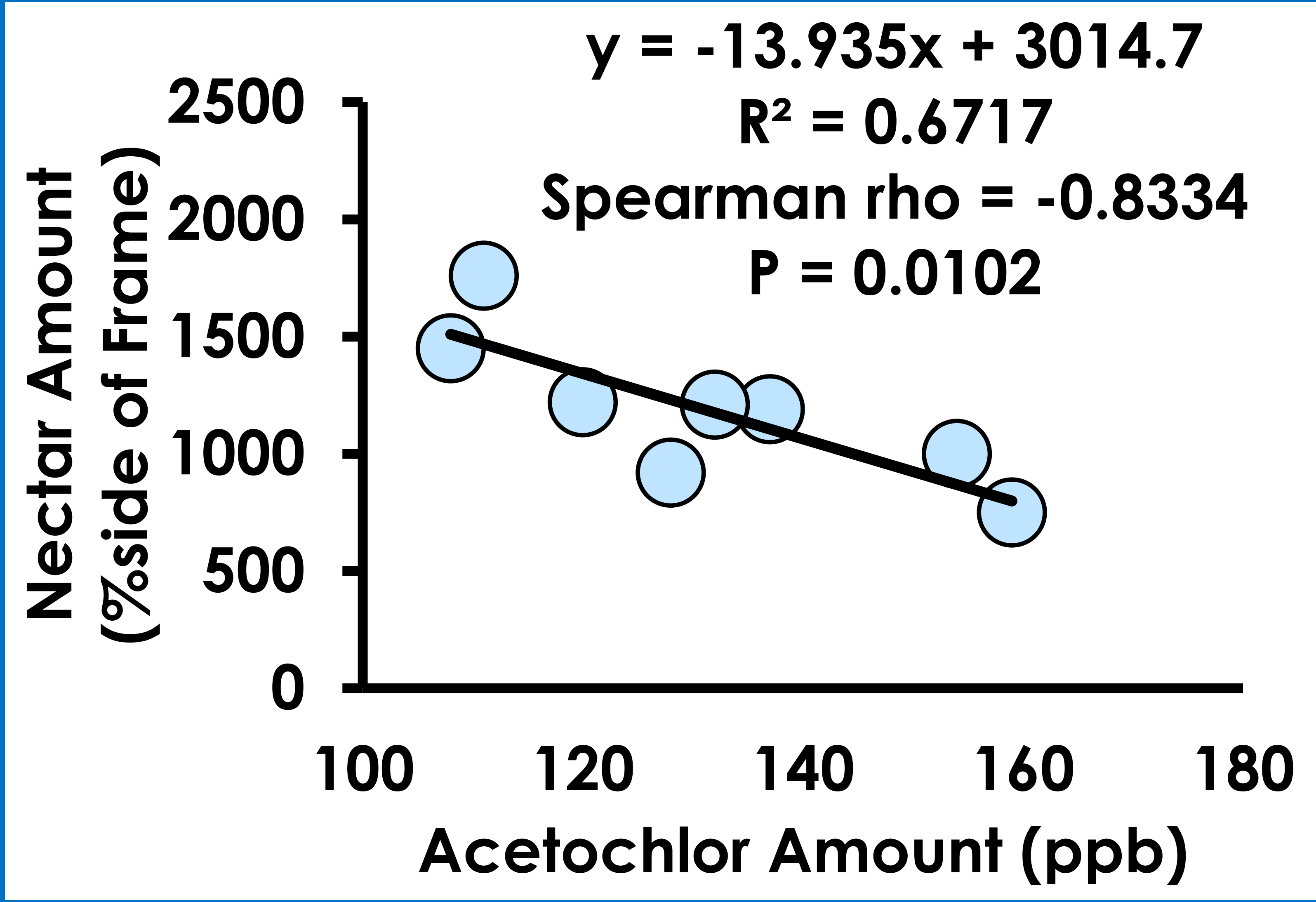
Fewer Frames of Bees with Increasing Number of Herbicides



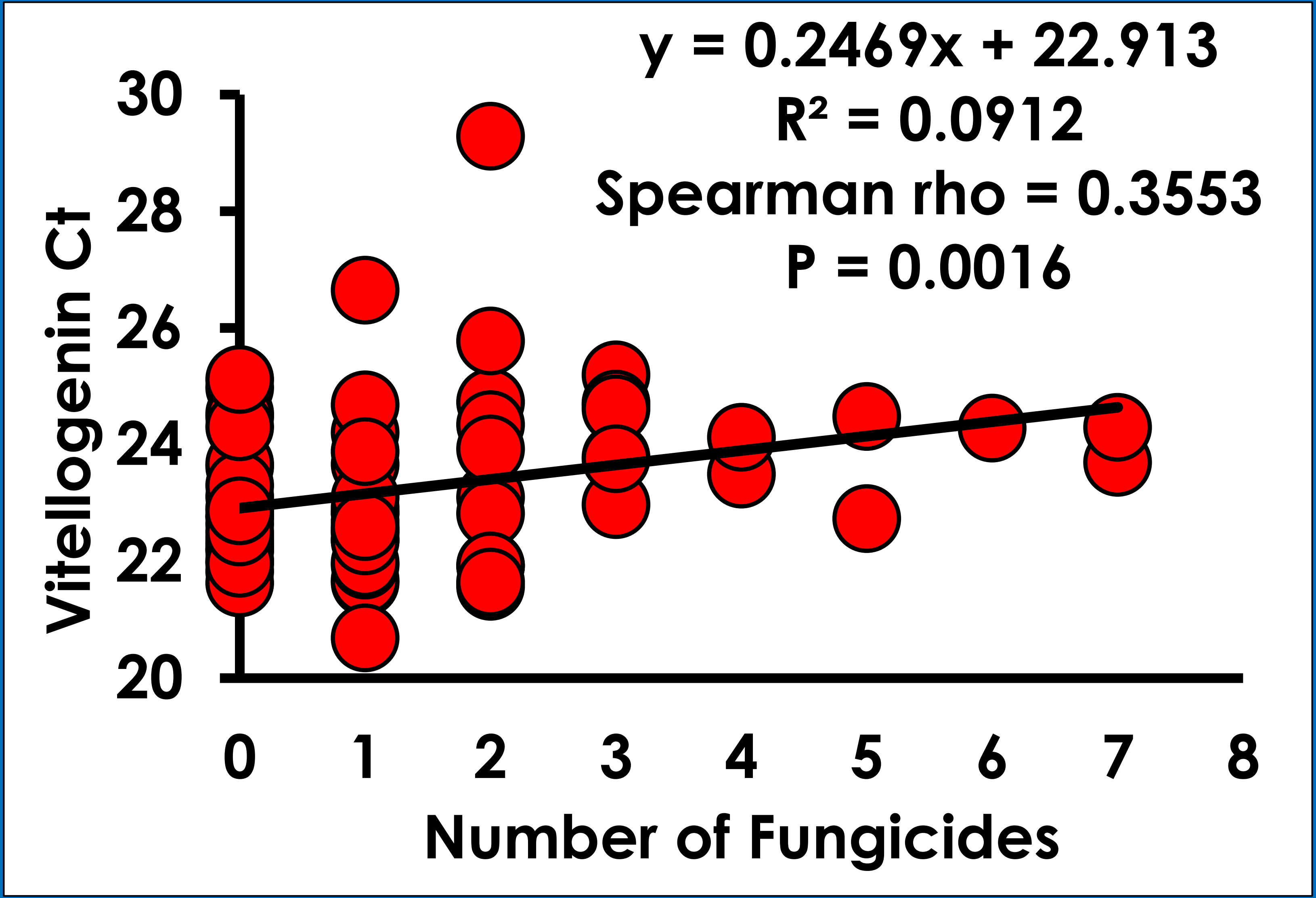
Less Pollen Area with Increasing Herbicide Amount



Less Nectar Area with Increasing Acetochlor Amount



Decreased Vitellogenin Expression with Increased Fungicide Number



Future Directions

- Year 2 sampling
- Effects of pesticide exposure on stress markers
  - ROS, lipid peroxidation, HSP,
- Epidemiological model development and validation

Conclusions

- No neonicotinoids were detected
- Pesticide exposure *in situ* is complex
- Synergistic interactions at low concentration may be prominent
- LC_{50}, LD_{50}, and HQ should be considered at the colony level
- Alternate endpoints besides mortality
- Validate field observations experimentally
- Significant effects are dwarfed by effects of *Varroa*

Acknowledgements

- **USDA-ARS**
  - Bob Danka
  - Mike Simone-Finstrom
  - Dave Dodge
  - Dan Winfrey
  - Victor Rainey
  - Garret Dodds
  - Bob Cox
  - Phil Tokarz
  - Hunter Martin
  - Natalie Martin

USDA NIFA

- **Louisiana State Univ.**
  - Kristen Healy (PI)
  - Daniel Swale
  - Hannah Penn
  - Thomas O'Shea-Wheller
  - Christopher Fellows
  - Sarah Lang
  - Sara Kennedy
- **Industry Partners**