Effects of neonicotinoids on honey bee queens and colony development

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Outline

Introduction

Indirect and sub-lethal effects of pesticides

Neonicotinoids & relevant exposure

Effects on honey bee queens

Cascading impacts on colony development

Integrating science into action

Pollination services

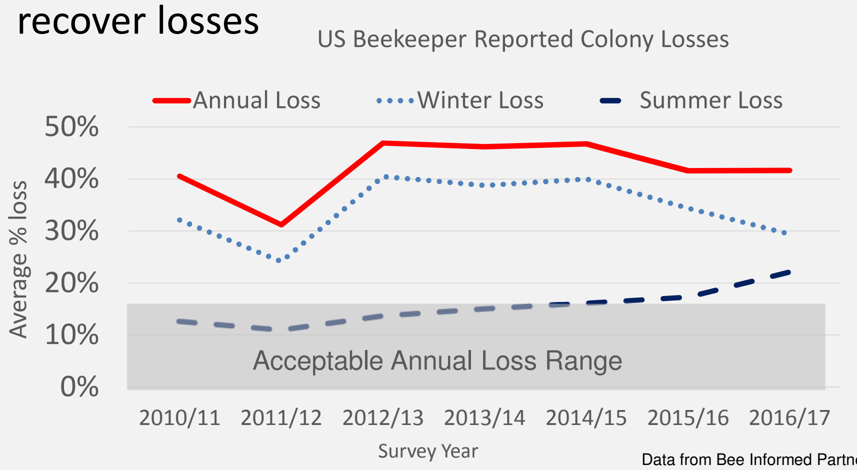
1/3 of our diet is pollinated by insects
$19 billion in US crop value
80% done by honey bees

Klein et al 2007; vanEngelsdorp et al 2010, 2011, 2012; Spleen et al 2013

Images: The Council of Canadians, steinershow.org

Honey bee populations are in decline

- US beekeepers have experienced unsustainable losses of colonies over the last decade
- Beekeepers must replenish colonies each year to recover losses

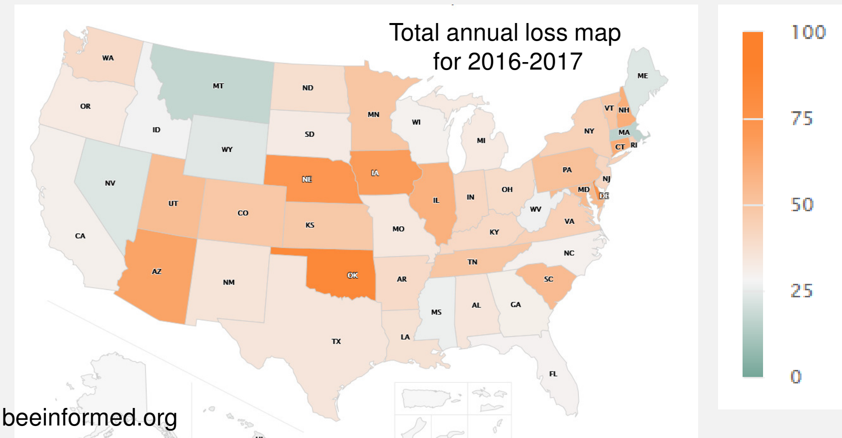


Data from Bee Informed Partnership

Bee informed Partnership (self reported losses)

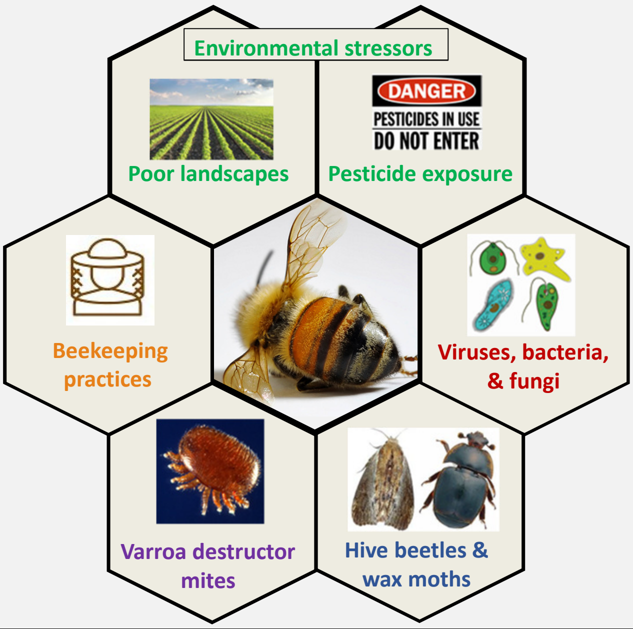
Winter loss in Nebraska: **56.7%**

Annual losses in Nebraska: **72.1%**



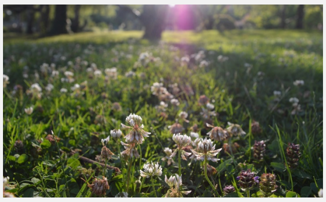
www.beeinformed.org

Multiple factors in bee health decline



Herbicides may indirectly impact bees

- Most are considered “non-toxic” to bees
- Used to remove weeds, however, most weeds are providing nectar/pollen for bees
- Reduces plant diversity which reduces nutritional diversity that is critical for bee health
- Some herbicides + carriers/surfactants can be toxic


Fungicides may directly or indirectly impact bees

- Do not target insects but may harm brood & adults
- Can kill beneficial fungi & disrupt conversion of pollen (↓nutritional value or absorption)
- May exhibit delayed effects
- May synergistically interact with insecticides and ↑toxicity of combination
- Used during bloom for many crops

Signs of entombed pollen?

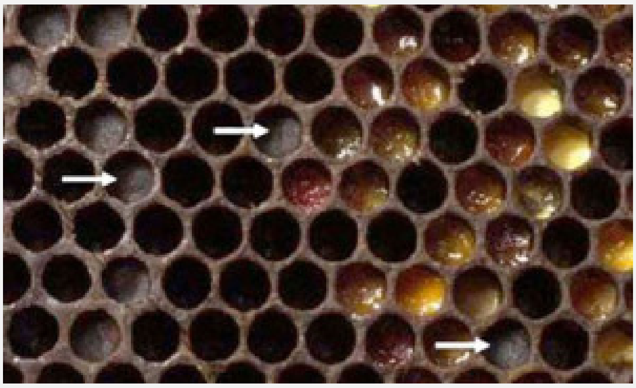
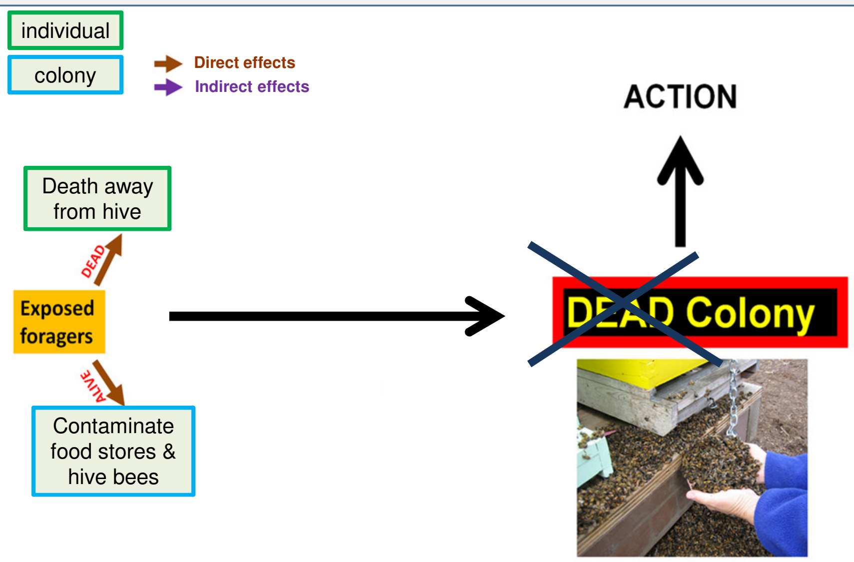


Photo Credit: D. vanEngelsdorp et al. / Journal of Invertebrate Pathology 101 (2009) 147–149

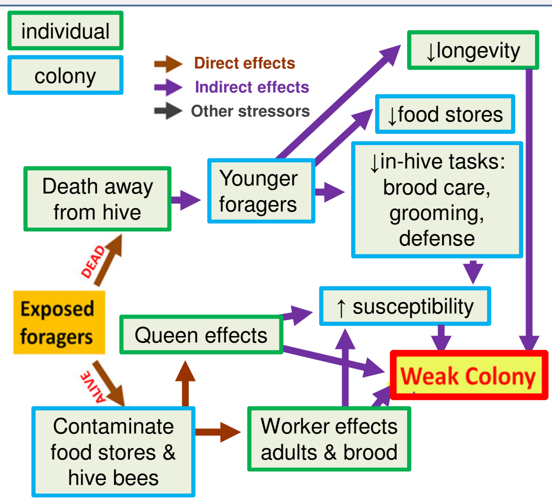
Pollen underneath has multiple compounds
Is full of pollen husks (no nutritional benefit)
High in fungicides (chlorothalonil)

How old is your comb?

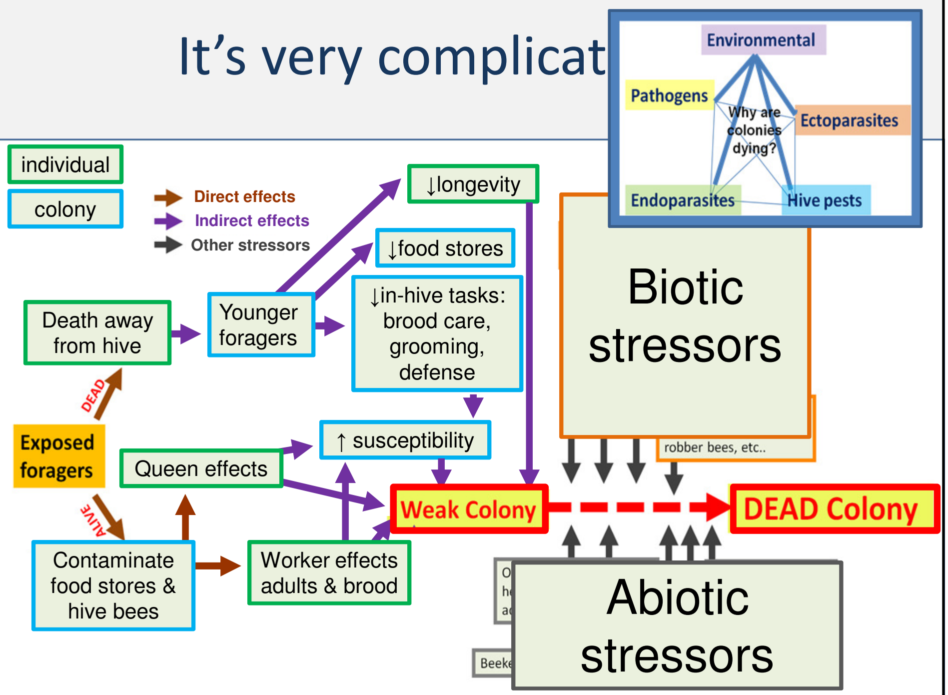
Effects of environmental insecticides



It’s very complicated



It’s very complicat



Potential effects of pesticides vary

Acute Lethal Exposure

Individual Bee Deaths

Colonies of Bees

Chronic Sub-Lethal Exposure

Behavior

Foraging
Learning
Memory
Orientation
Grooming

Development

Inability to grow
More susceptible to disease
Shorter lifespan

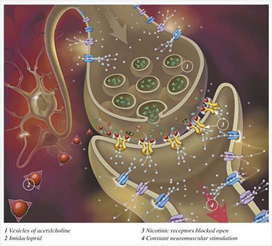
Reproduction

Improper mating
Reduced sperm viability
Reduced egg-laying
Queen/Drone development

Habitat

Reduced resource availability
Reduced diversity
Poor nutrition

Neonicotinoids and Bees



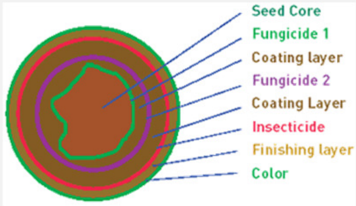
Mode of action:

- chloronicotinyl nitroguanidine/cyanoamidine insecticides
- binds nicotinic acetylcholine receptors (nAChR)
- constant transmission = paralysis & death
- higher affinity = ↑ insect selectivity
- systemic action: translocates to all parts of the plant (nectar/pollen)

Active ingredient:	Trade Name(s)
Imidacloprid	Merit, Marathon, Provado, Admire ….
Clothianidin	Poncho, Arena, Celero ….
Thiamethoxam	Centric, Cruiser, Flagship ….
Dinotefuran	Safari, Starkle, Abarin ….
Thiacloprid	Calypso, Bariard, Destroyer….
Acetamiprid	Transport, Assail, Chipco….

Methods of application

Foliar sprays



Seed-treatments

Soil drench

Trunk injection

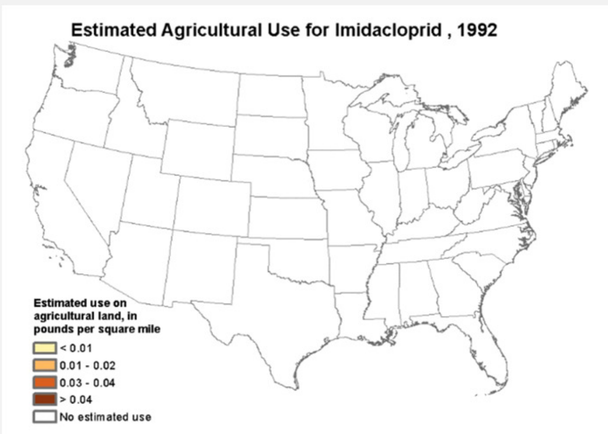
Chemgation

Images: UNL cropwatch, Masadour, chaitanyagroups.com, traxcoirrigation.com, valent.com, msue.anr.msu.edu

What are the issues?

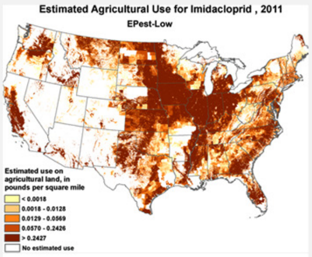
Challenges:

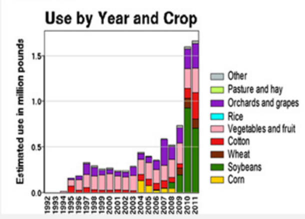
- Pervasive use and wide coverage
- Understanding environmental fate
- Estimating realistic/relevant neonicotinoid exposure



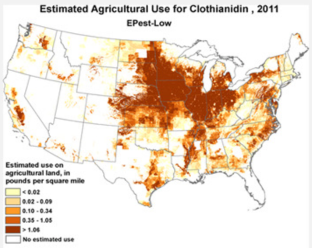
In 2011, 4.7 million pounds of the three neonicotinoids were used in the US

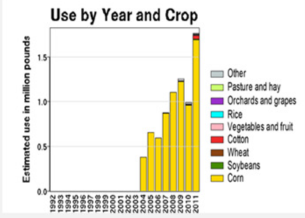
Imidacloprid



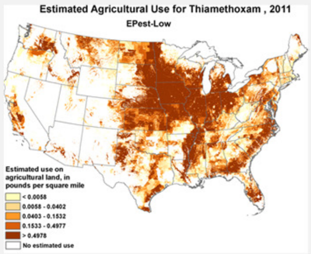


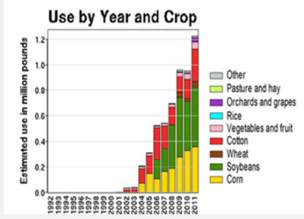
Clothianidin





Thiamethoxam

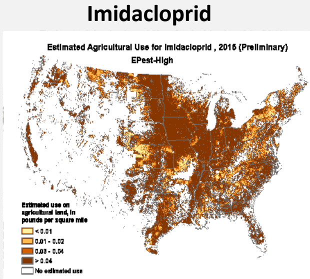


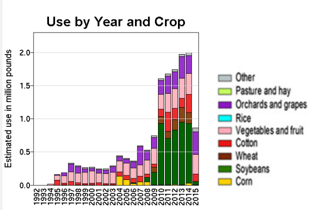


In 2015, <1.5 million pounds used?

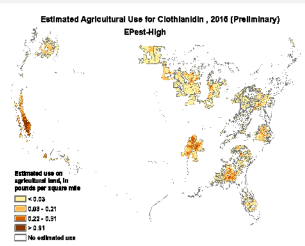
Beginning 2015, the provider of the surveyed pesticide data used to derive the county-level use estimates discontinued making estimates for seed treatment application of pesticides because of complexity and uncertainty. Pesticide use estimates prior to 2015 include estimates with seed treatment application.

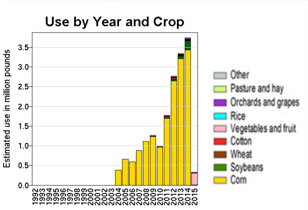
Imidacloprid



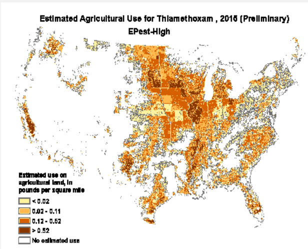


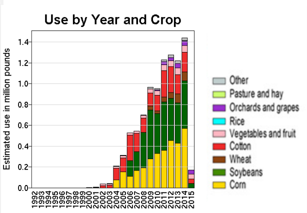
Clothianidin



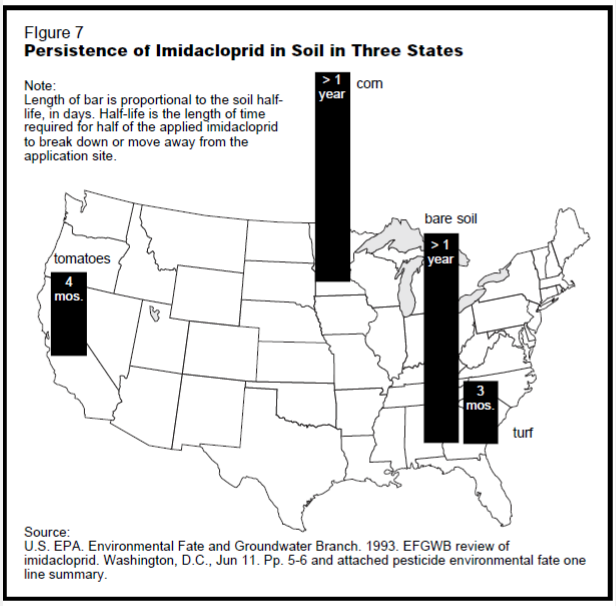


Thiamethoxam





Persistence & Bioactivity



Data lacking

Are they bioactive?
in soil?
in water?

Can residues be continuously taking up by roots?

Estimating exposure: Imidacloprid

App	Residue levels	Reference
Seed	0.6-1.9 ppb sunflower & corn (pollen); canola (nectar)	Schmuck et al. 2001 Bonmatin et al. 2005 Scott-Dupree & Spivak 2001
Soil	3-10 ppb purple tansy (nectar)	Wallner et al. 1999
Soil	15-27 ppb buckwheat (nectar)	Krischik et al. 2007
Water	30-80 ppb pumpkin (pollen); 4-12 ppb pumpkin (nectar)	Dively & Hooks 2010
Soil	27-850 ppb rhododendron (blossom)	Doering et al. 2004
Soil	1,038-2,816 ppb cornelian cherry (blossom)	Doering et al. 2005

Estimating exposure: Imidacloprid

<10 20 40 60 80 100 200 400 800 1600+

Exposure rates (ppb): Seed-applied | Agricultural | Urban landscape

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Potential effects on honey bee queens

Wu-Smart, J. & Spivak, M. 2016. *Scientific Reports* 6 (32108)

Do neonicotinoids adversely affect egg-laying rate and activity?

Can colony size influence queen exposure and response?

-population buffer ?

Method:

One-frame:

Laying queen + ~***1500 workers*** fed 80 ml

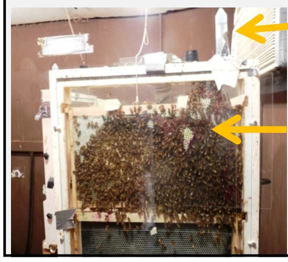
Two-frame:

Laying queen + ~***3000 workers*** fed 160 ml

Five-frame:

Laying queen + ~***7000 workers*** fed 320 ml

0, 10, 20, 50, 100 ppb imidacloprid in 50% sucrose



Treatment (every other day)

undrawn empty comb

Dose response at the colony level

Social Immunity

Structural defense: Queen and brood are centrally located

Population buffer: dilute pesticide levels

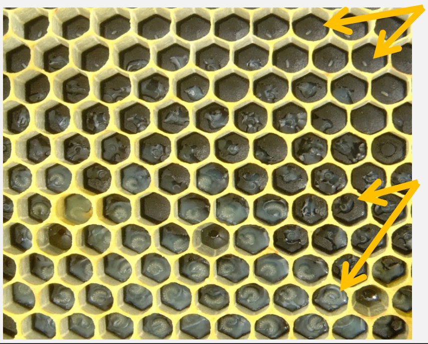
larger colonies = more foraging, food-sharing and grooming

Method:

From 2012-2014: colonies absconded or queens disappeared or died

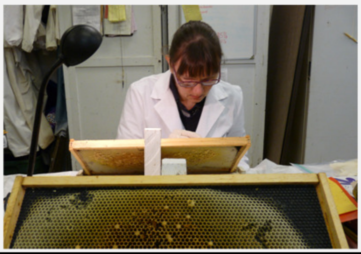
	0	10	20	50	100
1-frame	6	2	7	6	6
2-frame	6	2	8	6	6
5-frame	6	4	5	5	4
total	18	8	20	17	16

Newly drawn comb with brood



eggs

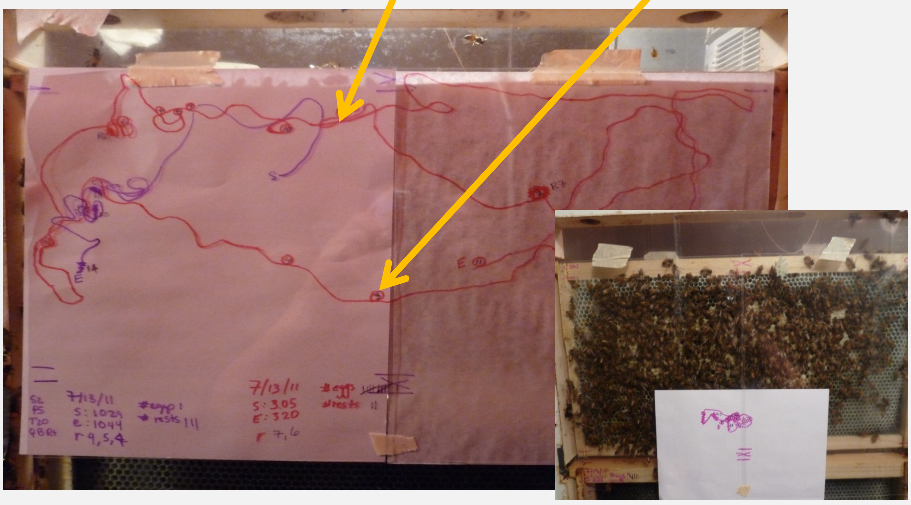
larvae



Tracking queen egg-laying & activity

Two 15 min observations (am/pm) every day (1 & 2- frame) or every other day (5-frame) over 3 weeks:

marked queen activity (path and position of eggs laid)



Measurements (~ 3 weeks):

- queen egg-laying rate (average # eggs laid)
- queen activity (average distance traveled)
- queen inactivity (time spent resting)
- worker hygienic behavior (in-hive activity)
- worker foraging (1 min counts 2x day)

***post-experiment assessment (after 23 days)*:**

- brood production (eggs, larvae, pupae) & pattern
- nectar & pollen stores
- adult population

Queen egg-laying

Measures during chronic exposure

For all measures taken during exposure:

Repeated measures ANOVA: (dose, size, and time)

***Time*:** Data collected everyday (1- & 2-frames) or every other day (5-frame) so data combined by week
Effects were strong in week 1, generally sustained in weeks 2 & 3

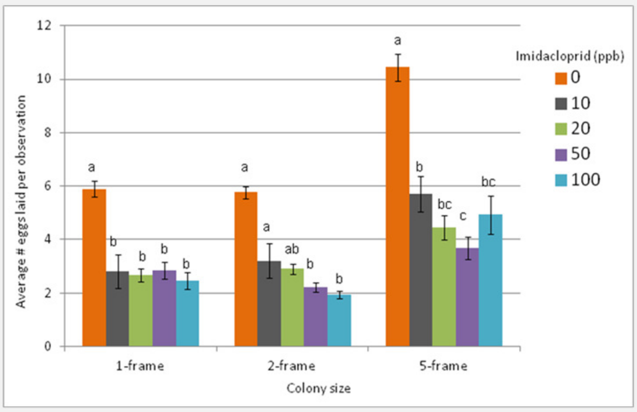
No interaction effect with dose & size therefore data was pooled over weeks

Size: 5-frame colonies were sig diff in all measures than 1 & 2 frames hives
BUT no differences between 1 & 2 frames

Measures during chronic exposure

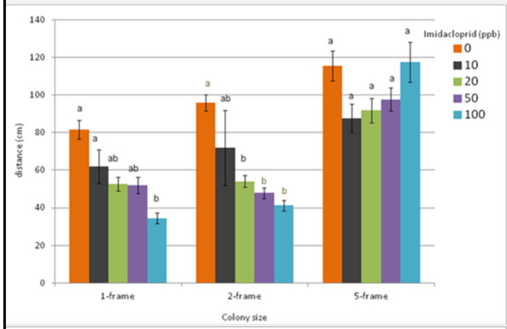
Queen egg-laying:
Dose: (F=100.9; df=4, 1083; p<2e-16)

Control laid significantly more eggs in all hives all weeks



Different letters denote significance (α=0.05) ***within*** a colony size

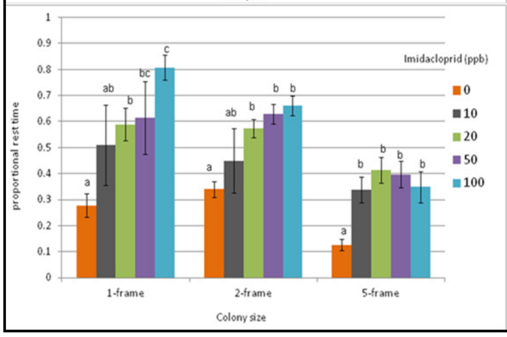
Measures during chronic exposure



Queen activity (distance):
Dose: (F=4.5; df=4, 2183; p=1.3e-3)

Controls traveled significantly more in 1- & 2- frames only

5-frames periods of hyperactivity?



Queen inactivity (time spent resting):
Dose: (F=67.9; df=4, 1213; p< 2e-16)

Controls rested significantly less at all colony sizes

Different letters denote significance (α=0.05) ***within*** a colony size

In-hive activity

Assessed using Hygienic Behavior assay on 5-frame colonies only

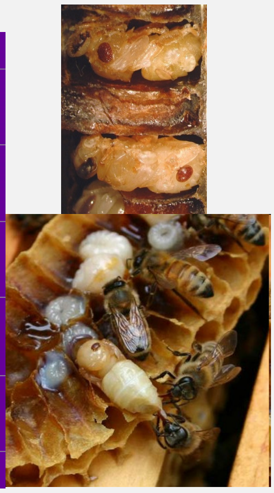
Hygienic Behavior Assay

Rate of removal of dead brood is correlated with rate of removal of diseased and mite infested brood

Ability of bees to detect and remove diseased or mite-infested brood,

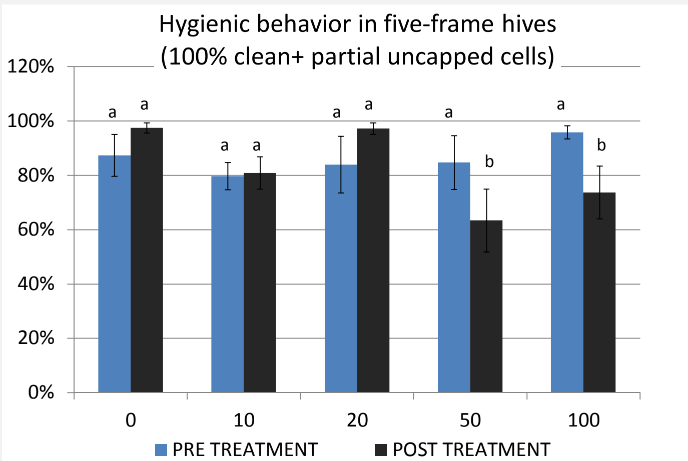
BEFORE disease forms infectious spores and

AFTER mite has started egg-laying



Park et al. 1937; Woodrow 1942; Rothenbuhler 1964; Spivak, 1996

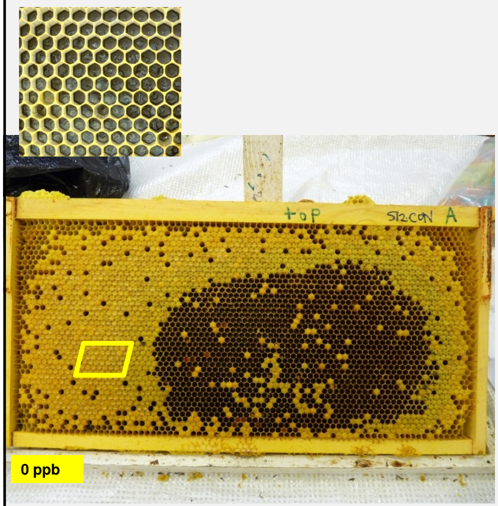
Hygienic Behavior Assay

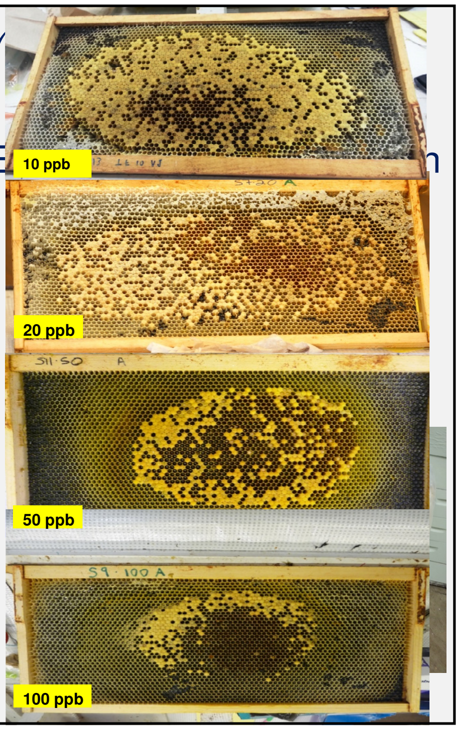


PRE: F=0.3; df=4,18; p=0.9 POST: F=4.5; df=4, 18; p=0.01

Use hygienic behavior as a measure of “in-hive worker activity”

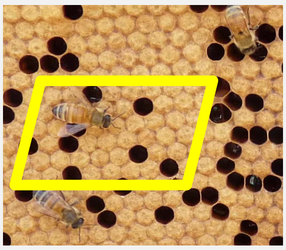
Post experiment (

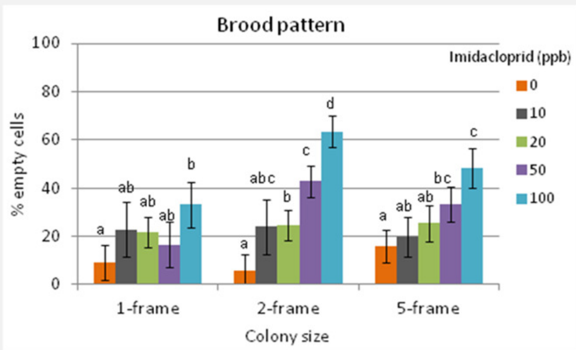




Post experiment measures

Brood pattern = indicator of quality of brood care & brood health





Dose-dependent effect on brood pattern

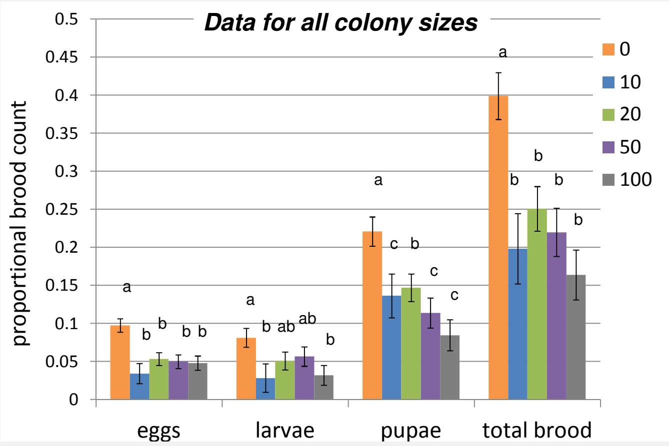
No differences between the three colony sizes

Dose effect: ($F=11$, $df=4$, $p<0.0001$)
colony size effect: ($F=2.1$, $df=2$, $p=0.14$)

Different letters denote significance ($\alpha=0.05$) ***within*** a colony size

Post experiment measures

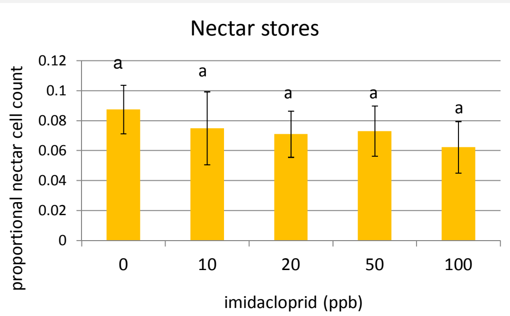
Brood production:
Controls had sig more brood after 3 weeks of imidacloprid exposure



Eggs (df=4, 74; p=0.0002) Larvae (df=4, 74; p=0.0568)
Pupae (df=4, 74; p=0.0001) Total brood (df=4, 74; p<0.0001)

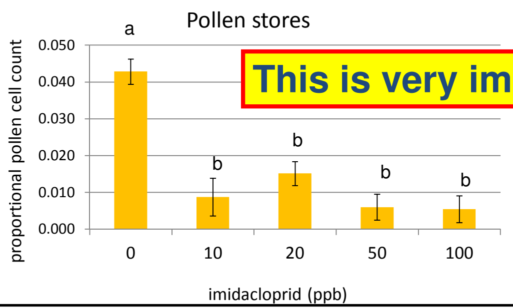
Different letters denote significance (α=0.05) ***within*** each group

Post experiment measures



Data for all colony sizes

Nectar (df=4, 74; p=0.8783)



This is very important!

Pollen (df=4, 74; p<0.0001)

Sub-lethal effects of dietary neonicotinoids on honey bee queen fecundity and colony development

Untreated queens laid more eggs & were more active
(traveled more,rested less)

Foraging activity was lower in all treated colonies = ↓ out-hive activity
(*not shown*)

Hygienic behavior was lower (50, 100ppb) = ↓ in-hive activity

Brood production and pollen stores were lower in all treated hives

No differences among treatments in final adult worker population (*not shown*)

Some indication that population can act to "buffer" exposure
= response less severe as size ↑

"buffer"= more social interactions (food sharing & grooming) to dilute toxicant

Do neonicotinoids adversely affect egg-laying rate & activity in honey bee queens?

Can colony size influence queen exposure and response?

Yes, effects on queens were observed at all doses but some responses lessened with increasing population

?

Integrating the science

Early spring management:

- Colonies can be small coming out of winter
- “split” or divide over-wintered colonies in the spring
- purchase “packages” (7,000-10,000 bees) to restock dead colonies

Recommendation:

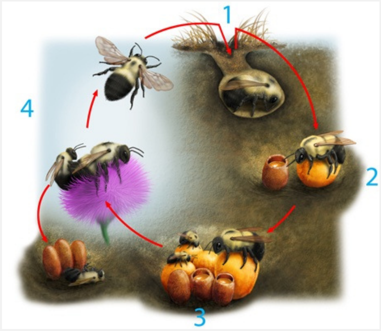
- reduce exposure risks in the early spring when honey bee colonies are at their smallest population size and when queens are more vulnerable.
- Plant more early spring forage to dilute potential contaminated sources

What does it all mean?

- It’s complicated and more research is needed
- Effects are wide ranging and linkages incomplete
- Weight-of-evidence is greater for individual-level effects when exposure levels are high (ex. **dusts** & foliar sprays) and soil drench? chemgation?
- Exposure studies are desperately needed to relate effects studies

- Early spring exposures represent greater risks to honey bee queens and bumble bee queens (**dust**)

Always social

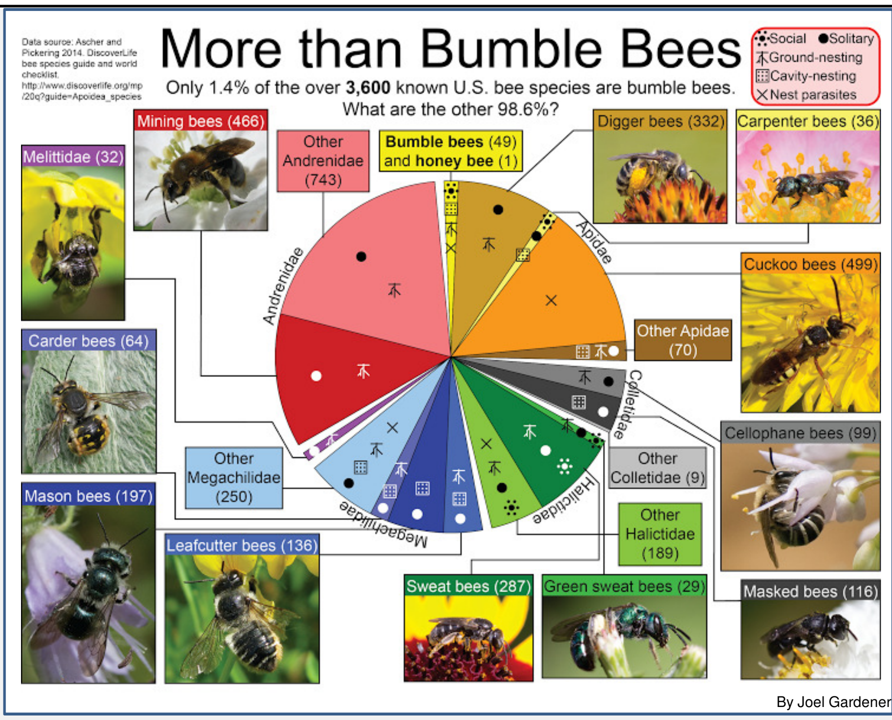


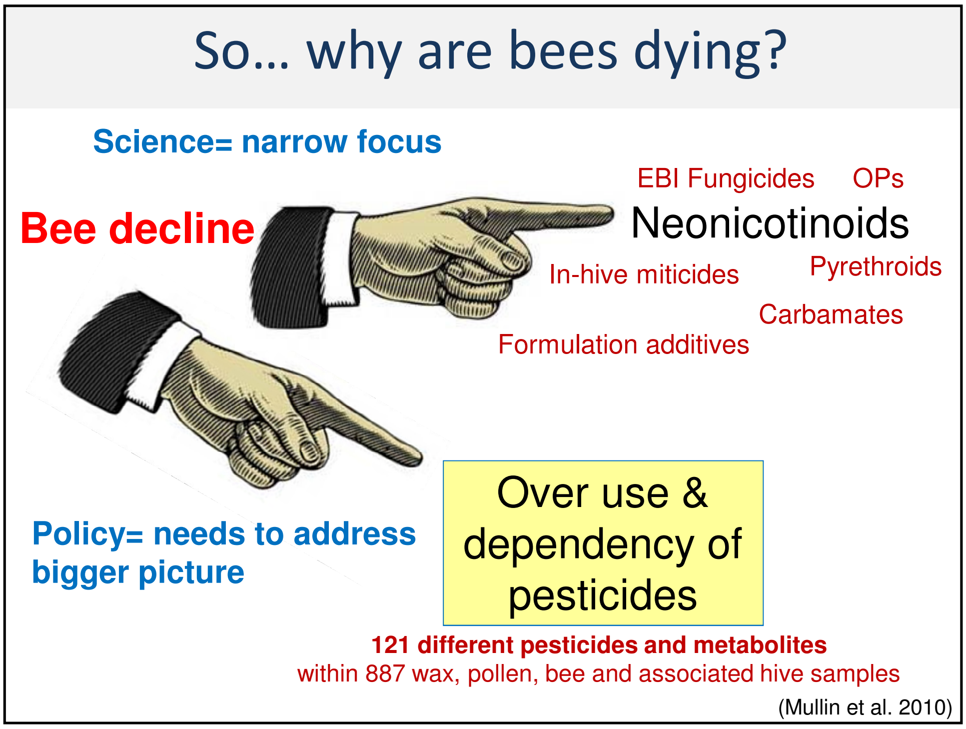
Has solitary phase

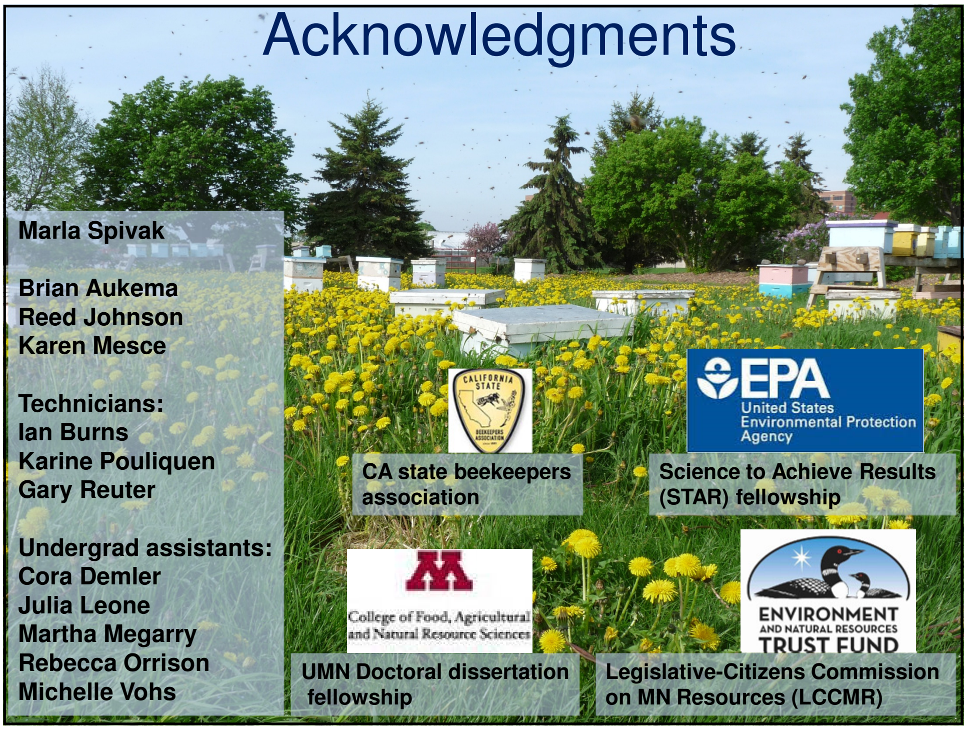
What about solitary bees?

- Can we identify other time points/conditions that put pollinators at greater risk?

To be continued.......




So… why are bees dying?
Science= narrow focus
Bee decline
EBI Fungicides
OPs
Neonicotinoids
In-hive miticides
Pyrethroids
Carbamates
Formulation additives
Policy= needs to address bigger picture
Over use & dependency of pesticides
121 different pesticides and metabolites
within 887 wax, pollen, bee and associated hive samples
(Mullin et al. 2010)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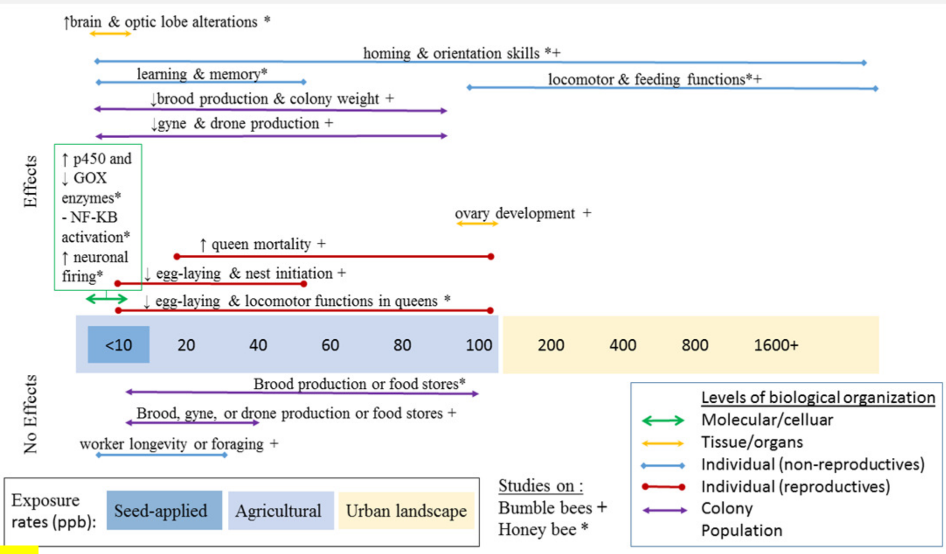
Relating effects to relevant exposures



51