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


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

US Beekeeper Reported Colony Losses

Annual Loss  Winter Loss  Summer Loss

Acceptable Annual Loss Range

Data from Bee Informed Partnership
Bee informed Partnership (self reported losses)

Winter loss in Nebraska: **56.7%**
Annual losses in Nebraska: **72.1%**

Multiple factors in bee health decline

- Environmental stressors
  - Poor landscapes
  - Pesticide exposure
- Beekeeping practices
- Varroa destructor mites
- Hive beetles & wax moths
- Viruses, bacteria, & fungi
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?**

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

**How old is your comb?**

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**Effects of environmental insecticides**

- **Direct effects**
- **Indirect effects**

- Death away from hive
- Exposed foragers
- Contaminate food stores & hive bees

*Photo Credit: D. vanEngelsdorp et al. / Journal of Invertebrate Pathology 101 (2009) 147–149*
It’s very complicated

<table>
<thead>
<tr>
<th>Individual</th>
<th>Colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct effects</td>
<td>Indirect effects</td>
</tr>
<tr>
<td>Other stressors</td>
<td></td>
</tr>
</tbody>
</table>

**Death away from hive**

**Younger foragers**

**Queen effects**

**Contaminate food stores & hive bees**

**Worker effects adults & brood**

**↓**

**Food stores**

**↓**

**In-hive tasks: brood care, grooming, defense**

**↑**

**Susceptibility**

**↓**

**Longevity**

**Weak Colony**

**Exposed foragers**

**Contaminate food stores & hive bees**

**Worker effects adults & brood**

**↓**

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**In-hive tasks: brood care, grooming, defense**

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**Susceptibility**

**↓**

**Longevity**

**Weak Colony**

**Biotic stressors**

**Abiotic stressors**

**Environmental**

**Pathogens**

**Endoparasites**

**Ectoparasites**

**Hive pests**

**DEAD Colony**
Potential effects of pesticides vary

**Acute Lethal Exposure**
- Individual Bee Deaths
  - Behavior
  - Foraging
  - Learning
  - Memory
  - Orientation
  - Grooming

**Chronic Sub-Lethal Exposure**
- Colonies of Bees
- Behavior
- Development
- Reproduction
- Habitat

**Inability to grow**
- More susceptible to disease
- Shorter lifespan

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

**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:**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Trade Name(s)</th>
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<tbody>
<tr>
<td>Imidacloprid</td>
<td>Merit, Marathon, Provado, Admire</td>
</tr>
<tr>
<td>clothianidin</td>
<td>Poncho, Arena, Celero</td>
</tr>
<tr>
<td>thiamethoxam</td>
<td>Centric, Cruiser, Flagship</td>
</tr>
<tr>
<td>dinotefuran</td>
<td>Safari, Starkle, Abarin</td>
</tr>
<tr>
<td>thiacycloprid</td>
<td>Calypso, Bariard, Destroyer</td>
</tr>
<tr>
<td>acetamiprid</td>
<td>Transport, Assail, Chipco</td>
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17/09/2018
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

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.
Persistence & Bioactivity

Data lacking
Are they bioactive in soil? in water?
Can residues be continuously taking up by roots?

Estimating exposure: Imidacloprid

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<tr>
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<th>Residue levels</th>
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<td>Seed</td>
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<tr>
<td>Water</td>
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Potential effects on honey bee queens


Do neonicotinoids adversely affect egg-laying rate and activity?
Can colony size influence queen exposure and response?
-population buffer?

Free-flying colonies have access to outside forage
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

Social Immunity

Structural defense: Queen and brood are centrally located
Population buffer: dilute pesticide levels
larger colonies = more foraging, food-sharing and grooming
From 2012-2014:

<table>
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<th>Method:</th>
<th>colonies absconded or queens disappeared or died</th>
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<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1-frame</td>
<td>6</td>
</tr>
<tr>
<td>2-frame</td>
<td>6</td>
</tr>
<tr>
<td>5-frame</td>
<td>6</td>
</tr>
<tr>
<td>total</td>
<td>18</td>
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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
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.

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Measures during chronic exposure

**Queen egg-laying:**

**Dose:** \( F=100.9; \text{df}=4, 1083; p<2e-16 \)

Control laid significantly more eggs in all hives all weeks.

![Graph showing egg-laying differences across different doses and colony sizes.](graph.png)

Different letters denote significance \((\alpha=0.05)\) \textit{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 (\( \alpha=0.05 \)) within a colony size.

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**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

Hygienic behavior in five-frame hives (100% clean + partial uncapped cells)

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 (after 3 week exposure)

Quantify all cells with:
- eggs (open cell)
- larvae (open cell)
- pupae (capped cell)
- pollen

Brood pattern: determined by empty cell count

Dose effect: (F=11, df=4, p<0.0001)

Colony size effect: (F=2.1, df=2, p=0.14)

Dose-dependent effect on brood pattern

No differences between the three colony sizes

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

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

Dose effect: (F=11, df=4, p<0.0001)

Colony size effect: (F=2.1, df=2, p=0.14)
Post experiment measures

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

Data for all colony sizes

Proportional brood count

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

This is very important!

Nectar stores

Pollen stores

Data for all colony sizes

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

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**)

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

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*What about solitary bees?*

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**More than Bumble Bees**

Only 1.4% of the over 3,600 known U.S. bee species are bumble bees. What are the other 98.6%?

*By Joel Gardener*
So... why are bees dying?

Science= narrow focus

Bee decline

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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