ARE HONEY BEES NEGATIVELY AFFECTED BY PESTICIDES IN THE HIVE?

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ECONOMIC IMPACT

- 148 million lbs. of honey produced by 2.49 million colonies in US (NASS 2012). The net worth was 256 million USD.
- 15 billion USD added to agriculture industry nationally.
- 10-20% of world’s food production is dependent on honey bees
Number of honey producing colonies in the United States (NASS 2009).

Ellis, Evans, Pettis, 2010, JAR 49(1): 134-136
The Possible Culprits

1) traditional bee pests/diseases
2) how the bees were managed
3) queen source
4) genetically modified crops ("Frankenfoods")
5) *Varroa* mites
6) inadequate nutrition
7) chemical use in bee colonies
8) chemical toxins in the environment
9) climate stress (some call this environmental stress)
10) undiscovered (or newly discovered) bee pests/pathogens
The Possible Culprits - Pesticidal

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Chemical use in bee colonies, both label and off-label use
Chemical toxins in the environment

Honey Bees:
- visit flowers of all kinds
- visit bird feeders, animal feed bins, etc.
- visit bird baths, pools, streams, rivers, ponds, lakes
- have been known to collect paint, glue, any sticky substance, etc.
As honey bees forage:

A circle with a 5-8 km radius encompasses ~79 – 200 kilometers²

http://burnsandburnsmiami.com/BeeFacts.aspx
Rearing Bioassay

Determining LC50 values for 9 pesticides affecting larval honey bees
Determine pesticide effects on larvae cell death

Midgut epithelium cells of coumaphos-treated larva – ISDDK technique

Ales Gregorc
Table 1. Cell mortality determined using two TUNEL kits, DeadEnd and ISCDDK. Data are mean ± s.e. proportion cells positively stained, N out of at least 300 cells counted from a minimum of 3 larvae. When both treatments were analyzed together, neither technique (DeadEnd or ISCDDK - $F = 1.1$; df = 1,123; $P = 0.29$) nor the interaction between technique and treatment ($F = 1.6$; df = 10,123; $P = 0.13$) affected the proportion of cells positively stained. Columnar data followed by the same letter are not different at $P < 0.05$. Students T tests were used to compare means.

<table>
<thead>
<tr>
<th>Type of pesticide</th>
<th>Treatment ↓</th>
<th>Dead End technique</th>
<th>ISDDK technique</th>
<th>Both techniques analyzed together</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insecticide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.56 ± 0.08, 6abc</td>
<td>0.74 ± 0.04, 6a</td>
<td>0.65 ± 0.05, 12abc</td>
<td></td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>0.51 ± 0.12, 6bcd</td>
<td>0.76 ± 0.02, 4a</td>
<td>0.61 ± 0.08, 10abc</td>
<td></td>
</tr>
<tr>
<td>Amitraz*</td>
<td>0.64 ± 0.07, 5abc</td>
<td>0.37 ± 0.03, 4c</td>
<td>0.52 ± 0.06, 9c</td>
<td></td>
</tr>
<tr>
<td>Fluvalinate*</td>
<td>0.29 ± 0.04, 7d</td>
<td>0.32 ± 0.02, 3c</td>
<td>0.3 ± 0.03, 10d</td>
<td></td>
</tr>
<tr>
<td>Coumaphos*</td>
<td>0.58 ± 0.12, 8abc</td>
<td>0.48 ± 0.03, 5bc</td>
<td>0.54 ± 0.07, 13c</td>
<td></td>
</tr>
<tr>
<td><strong>Fungicide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>0.62 ± 0.11, 5abc</td>
<td>0.78 ± 0.06, 4a</td>
<td>0.69 ± 0.07, 9ab</td>
<td></td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>0.49 ± 0.08, 7cd</td>
<td>0.66 ± 0.06, 4ab</td>
<td>0.55 ± 0.06, 11bc</td>
<td></td>
</tr>
<tr>
<td><strong>Herbicide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyphosate</td>
<td>0.74 ± 0.05, 5ab</td>
<td>0.65 ± 0.15, 5a</td>
<td>0.69 ± 0.08, 10ab</td>
<td></td>
</tr>
<tr>
<td>Simazine</td>
<td>0.76 ± 0.06, 7a</td>
<td>0.77 ± 0.06, 7a</td>
<td>0.77 ± 0.04, 14a</td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet with acetone</td>
<td>0.10 ± 0.01, 8e</td>
<td>0.11 ± 0.03, 5d</td>
<td>0.11 ± 0.01, 13e</td>
<td></td>
</tr>
<tr>
<td>Diet only</td>
<td>0.10 ± 0.02, 8e</td>
<td>0.09 ± 0.02, 5d</td>
<td>0.10 ± 0.02, 13e</td>
<td></td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td>$F = 10.6$; df = 10,71; $P &lt; 0.01$</td>
<td>$F = 16.3$; df = 10,51; $P &lt; 0.01$</td>
<td>Treatment effect: $F = 22.2$; df = 10,123; $P &lt; 0.01$</td>
<td></td>
</tr>
</tbody>
</table>

*Used in honey bee colonies to control Varroa mites
Determine detoxification gene activity *in vitro*

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Solubility</th>
<th>Treatment Concentrations:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Amitraz</td>
<td>acetone</td>
<td>181&lt;sup&gt;A&lt;/sup&gt; pollen</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>acetone</td>
<td>1545&lt;sup&gt;A&lt;/sup&gt; wax</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>acetone</td>
<td>33&lt;sup&gt;A&lt;/sup&gt; wax</td>
</tr>
<tr>
<td>Chlothianidin</td>
<td>acetone (15.2g/L)</td>
<td>18.33&lt;sup&gt;C&lt;/sup&gt; pollen</td>
</tr>
<tr>
<td>Coumaphos</td>
<td>acetone</td>
<td>730&lt;sup&gt;A&lt;/sup&gt; pollen</td>
</tr>
<tr>
<td>Fluvalinate</td>
<td>acetone</td>
<td>294&lt;sup&gt;A&lt;/sup&gt; pollen</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>water (12g/L)</td>
<td>0.8&lt;sup&gt;D&lt;/sup&gt; pollen</td>
</tr>
<tr>
<td></td>
<td>(mean)</td>
<td>(max)</td>
</tr>
<tr>
<td>Simazine</td>
<td>chloroform (0.9 g/L)</td>
<td>0.8&lt;sup&gt;A&lt;/sup&gt; pollen</td>
</tr>
</tbody>
</table>

A: (Mullin et al, 2010) 95th percentile except as noted
B: (Wu, Anelli, Sheppard, 2011) average of detected concentrations
C: (Krupke et al 2012) average of detected concentrations
D: Glyphosate values matched to Simazine values, because both are systemic herbicides
in vitro reared prepupae
in vitro reared adults
Pesticidal effects on honey bee longevity

- Cages treated with 11 different pesticides at field-relevant concentrations
  - Amitraz, clothianidin, coumaphos, imidacloprid, glyphosate, bosalid, fluvalinate, simazine, chlorothalonil, systhane, and chlorpyrifos
  - Wax, pollen, or both
- Measured honey bee longevity until 100% mortality reached in all cages
Lab studies investigating stressor interactions
Effect of commercial diets on pesticide sensitivity and Nosema infection

- **Nosema** - microsporidian parasite

- **Objective 1:** Do certain pollen substitutes impact Nosema virulence
  - Little is known on how diet affects Nosema infection in honey bees. (Mattila and Otis 2006 showed no difference)

- **Objective 2:** What is the role of commercially available pollens on pesticide sensitivity
  - Previous findings show that bees fed a high-protein pollen diet are less sensitive to chlorpyrifos exposure (Schmehl et al. 2013, in prep.)
Nosema/nutrition pilot

- Nosema infection higher to bees fed wildflower pollen (confirms earlier findings by Rinderer and Elliott 1977)
- Pollen inoculation increased Nosema infection in bees
Interactive effects of pesticide exposure and *Nosema* infection on learning

- Chronic pesticide exposure at 95th percentile for pollen
  - Chlorothalonil, Coumaphos, Fluvalinate, Imidicloprid, Boscalid, Pyraclostrobin
- Infected with *Nosema* sp.
Pesticide exposure and *Nosema* infection on learning

- Proboscis Extension Response (PER) assay to test learning ability in forager age bees.
Are Native Pollinators at a Greater Risk?

Jason Graham, HBREL
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