The Role of Pesticides in Honeybee Decline

Brian Eitzer

Department of Analytical Chemistry
The Connecticut Agricultural Experiment Station
## Economic Value of Pollinators

<table>
<thead>
<tr>
<th>Crop Category</th>
<th>Worldwide Value ($ Billions)</th>
<th>Pollinator Value Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuts</td>
<td>20</td>
<td>31</td>
</tr>
<tr>
<td>Fruits</td>
<td>307</td>
<td>23</td>
</tr>
<tr>
<td>Edible Oil Crops</td>
<td>336</td>
<td>16</td>
</tr>
<tr>
<td>Vegetables</td>
<td>585</td>
<td>12</td>
</tr>
<tr>
<td>Stimulant Crops</td>
<td>27</td>
<td>39</td>
</tr>
</tbody>
</table>

Source: Gallai et al. in Ecological Economics (2009)
The Number of Honey Producing Colonies is Decreasing

Source: vanEngelsdorp and Meixner in Journal of Invertebrate Pathology (2010)
What are Possible Causes of Decline in Honey Bees?

– Migratory Stress
– Poor Nutrition
– New Diseases
– Varroa Mites
– Colony Collapse Disorder
– Pesticides
Pesticide Questions

• What is the toxicology of pesticide exposure?
  – Acute vs. Chronic (sub-lethal) Effects
  – Synergistic Effects

• What pesticides are honey bees exposed to?

• How does the exposure occur?

• How much of the various pesticides are they exposed to?

• How does the exposure change with time and location?

• Can pesticide exposure be correlated with hive health?
Analytical Procedures for Pesticide Analysis
Extract with a Modified QuEChERS Procedure

- 5 g of pollen/bees plus:
  - 6 g magnesium sulfate
  - 1.5 g sodium acetate
  - 15 mL water
  - 15 ml acetonitrile
  - C-13 Alachlor and D-4 Imidacloprod I.S.

- Shake / Vortex
- Centrifuge
Extraction (cont.)

- Take 10 mL aliquot
- Combine with
  - 1.5 g magnesium sulfate
  - 0.5 g PSA
  - 0.5 g C-18 silica
  - 2 mL toluene
- Shake
- Centrifuge
- Concentrate 6 mL to 1 mL with nitrogen for LC/MS analysis
Detection of Imidacloprid at 10 PPB in Sample

Standard Selected Ions
MS/MS of m/z = 256
RT: 11.28
MA: 11565

Pollen Sample Selected Ions
MS/MS of m/z = 256
RT: 11.29
MA: 19638

CAES- Spring Open House 2011
Internal Standard Response Curve (no matrix present)

Imidacloprid

\[ Y = 0.0667594 \times X \quad R^2 = 0.9974 \]
CAES Studies

• Suspected Honey Bee Poisoning
• Measurement of Pesticide Residues in Squash Nectar and Pollen after Agricultural Application
• Monitoring of Pollen Collected by Foraging Honey Bees
  – Coordinated Agricultural Program: Stationary Apiary Project
  – Connecticut Study
Study of an Acute Poisoning

• A honey bee researcher at Purdue University noted sick and dying bees in apiaries near recently planted cornfields during dry and windy spring.

• Young bees dying not foragers.

• As most of the corn seed in the area is treated with a pesticide toxic to bees a poisoning incident was suspected.

• Samples of honey bees and honey bee collected pollen analyzed.
# Pesticides Found in Parts Per Billion

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Pollen 84</th>
<th>Bee 1-P</th>
<th>Bee 104-P</th>
<th>Bee 84-P</th>
<th>Bee 91-P</th>
<th>Bee 95-P</th>
<th>Bee 71-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clothianidin</td>
<td>21</td>
<td>4.4</td>
<td>7.6</td>
<td>5.0</td>
<td>3.4</td>
<td>3.5</td>
<td>n.d.</td>
</tr>
<tr>
<td>Atrazine</td>
<td>310</td>
<td>11</td>
<td>11</td>
<td>13</td>
<td>24</td>
<td>12</td>
<td>2.8</td>
</tr>
</tbody>
</table>

LD 50’s for Honey Bees
- Clothianadin .04 ug/bee
- Thiamethoxam .03 ug/bee
- Imidacloprid .018 ug/bee
- Atrazine > 1000ug/bee
Follow Up

• Collect pollen from hives surrounding fields being planted with either treated or untreated seed.

• Honey bee pollen from hives around field with treated seed had 0 – 88 PPB clothianadin while those around the untreated field had only 0 -13 PPB.

• Study will continue this spring with funding from the North American Pollinator Protection Campaign.
Measuring Neonicotinoid Residues in Squash Nectar and Pollen

When we apply systemic insecticides to soil or through irrigation at labeled rates, as a farmer would, how much do we find in the nectar and pollen of the plant?

Squash bees on pumpkin flower-Liz Andrews, UMass
What we did:

• Grew squash (summer squash in 2009, summer squash and winter squash in 2010) according to standard farming methods

• Applied neonicotinoid insecticides imidacloprid (Admire Pro®) and thiamethoxam (Platinum®)

• Two methods of application:
  – To seed hole in black plastic just before seeding
  – In drip irrigation to transplants 4-5 days after transplant

• Rates:
  – Admire Pro®: 10 fl. oz. per acre (labeled range = 7 -10.5 )
  – Platinum®: 8 fl. oz. per acre (labeled range = 5- 11)
Collecting pollen and nectar
What we found:

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Average Concentration in Pollen (Overall)</th>
<th>Average Concentration in Nectar (Overall)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloriprid</td>
<td>14 ppb ± 8</td>
<td>10 ppb ± 3</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>12 ppb ± 9</td>
<td>11 ppb ± 6</td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>
Use Pollen Collected by Honey Bees to Evaluate Exposure to Pesticides

Photo by: Kathy Keatley Garvey, UC Davis
Connecticut Study
Locations of Hives 2007 - 2010

1. Our offices in New Haven on the edge of the city, 2007 - 2010
2. Our experimental farm surrounded mostly by suburbs, 2007 - 2010
3. An orchard on the edge of a suburb with pollen collected only during the blooming season of apples and blueberries, 2007 and 2009
4. Another suburban site on the edge of a large agricultural area growing vegetable crops, 2007 – 2010
5. Mixed agricultural and industrial area, 2009 - 2010
Bee Pollen Collection
Overall Data by Year

<table>
<thead>
<tr>
<th>Year</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungicides</td>
<td>11</td>
<td>10</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Herbicides</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Insecticides</td>
<td>15</td>
<td>9</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Avg. # per sample</td>
<td>4.3</td>
<td>5.4</td>
<td>5.8</td>
<td>4.0</td>
</tr>
</tbody>
</table>
### Frequently Detected Pesticides

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>2007 n=101</th>
<th>2008 n=44</th>
<th>2009 n=59</th>
<th>2010 n=62</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coumaphos (A, I)</td>
<td>96</td>
<td>44</td>
<td>41</td>
<td>36</td>
</tr>
<tr>
<td>Carbaryl (I)</td>
<td>66</td>
<td>16</td>
<td>9</td>
<td>38</td>
</tr>
<tr>
<td>Phosmet (I)</td>
<td>38</td>
<td>15</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Atrazine (H)</td>
<td>34</td>
<td>24</td>
<td>31</td>
<td>21</td>
</tr>
<tr>
<td>Imidacloroprid (I)</td>
<td>30</td>
<td>23</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Dithiopyr (H)</td>
<td>13</td>
<td>34</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>Pendamethalin (H)</td>
<td>11</td>
<td>24</td>
<td>30</td>
<td>19</td>
</tr>
<tr>
<td>Carbendazim (F)</td>
<td>18</td>
<td>20</td>
<td>24</td>
<td>22</td>
</tr>
</tbody>
</table>
Detection Frequency Depends on Hive Location

• In 2009 the orchard location averaged 12.0 residues per sample; the average of the other four sites was 5.4 residues per sample.

• In 2009 all 4 samples from the orchard location had difenconazole and 3 of 4 had myclobutanil; neither of these pesticides were seen at any of the four other hive locations that year.
High Frequency is not the same as High Concentration
(2009 Data)

<table>
<thead>
<tr>
<th>Pesticide</th>
<th># Detec. (n = 59)</th>
<th>Max. Conc. PPB</th>
<th>Avg. Conc. PPB</th>
<th>Median Conc. PPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine (H)</td>
<td>32</td>
<td>15</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Phosmet (I)</td>
<td>30</td>
<td>540</td>
<td>44</td>
<td>7</td>
</tr>
<tr>
<td>Imidacloprid (I)</td>
<td>8</td>
<td>19</td>
<td>5.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Azoxystrobin (F)</td>
<td>8</td>
<td>55</td>
<td>18.3</td>
<td>8.8</td>
</tr>
<tr>
<td>Myclobutanil (F)</td>
<td>3</td>
<td>4190</td>
<td>1490</td>
<td>270</td>
</tr>
<tr>
<td>Trifloxystrobin (F)</td>
<td>5</td>
<td>160</td>
<td>42</td>
<td>22</td>
</tr>
</tbody>
</table>

A = acaricide, F = fungicide, H = herbicide, I = insecticide
Pesticide Concentrations Can Change Rapidly

Imidacloprid at Site #4

Conc. in PPB

Date

Hive Location
## Selected Pesticides in Sorted Pollen

<table>
<thead>
<tr>
<th>Pollen Color</th>
<th>Pollen Type(s)</th>
<th>Imid</th>
<th>Carb</th>
<th>Carben</th>
<th>Meth</th>
<th>Atr</th>
<th>Phos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark Brown</td>
<td>white clover</td>
<td>611</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>Reddish-Brown</td>
<td>alfalfa</td>
<td>199</td>
<td>n.d.</td>
<td>5.8</td>
<td>n.d.</td>
<td>2.1</td>
<td>6.3</td>
</tr>
<tr>
<td>Yellowish-Brown</td>
<td>black locust</td>
<td>114</td>
<td>n.d.</td>
<td>6.4</td>
<td>n.d.</td>
<td>3.8</td>
<td>6.9</td>
</tr>
<tr>
<td>Orange</td>
<td>ragweed</td>
<td>21</td>
<td>15</td>
<td>8.7</td>
<td>n.d.</td>
<td>38</td>
<td>18</td>
</tr>
<tr>
<td>Orange-Yellow</td>
<td></td>
<td>6.6</td>
<td>n.d.</td>
<td>5.5</td>
<td>8.4</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Medium Brown</td>
<td></td>
<td>3.9</td>
<td>182</td>
<td>n.d.</td>
<td>n.d.</td>
<td>16</td>
<td>55</td>
</tr>
<tr>
<td>Greenish-Yellow</td>
<td></td>
<td>3.1</td>
<td>23</td>
<td>n.d.</td>
<td>17</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Round</td>
<td></td>
<td>2.4</td>
<td>n.d.</td>
<td>n.d.</td>
<td>17</td>
<td>8.6</td>
<td>3.1</td>
</tr>
<tr>
<td>Pure-Yellow</td>
<td></td>
<td>1.7</td>
<td>13</td>
<td>2.4</td>
<td>n.d.</td>
<td>6.5</td>
<td>4.1</td>
</tr>
</tbody>
</table>

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Objective:

Identify causal factors or interactive effects of these factors (pests, pathogens and pesticides) in causing losses in stationary honey bee colonies across the United States.
Standardized Data Collection

I. weather conditions
II. landscape composition
III. pesticide contamination (pollen, wax)
IV. package source and queen genetic makeup
V. colony productivity and survival
  ➢ frames of adult bees and sealed brood
  ➢ egg laying and brood pattern quality
  ➢ queen status (presence / absence)
  ➢ supercedure
VI. infestation
  1. Varroa mites – mites per 280 adult bees
  2. SHB – adults and larvae
  3. Tracheal mite dissections
  4. Nosema (spp. ID and spore counts)
  5. chalk brood symptoms
  6. bacterial pathogens
  7. viral symptoms and molecular markers: DWV, IAPV, SBV, BQCV

MN, August 2009
CAP Stationary Apiaries, 2009

MN, August 2009

TX, November 2009

Taking Samples
Pesticides Vary with Sample Location and Time
(2009 Data)

Conc. (PPB)

Dimethoate
Atrazine

W-June
W-July
WAug
W-Sep
T-May
T-June
T-July
T-Aug
T-Sep
P-June
P-July
P-Aug
MN-May
MN-June
MN-July
MN-Aug
MN-Sep
ME-April
ME-May
ME-June
ME-July
ME-Aug
ME-Sep
F-May
F-June
F-July
F-Aug
F-Sep
F-Oct

181
Pollen Samples Differ by Hive Even at Same Location and Date
Compositing vs. Individual Samples

- **Phosmet**
- **Dimethoate**

<table>
<thead>
<tr>
<th>Date</th>
<th>Concentration (PPB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 5</td>
<td>181</td>
</tr>
<tr>
<td>June 19</td>
<td>220</td>
</tr>
<tr>
<td>June 24</td>
<td>945</td>
</tr>
<tr>
<td>June 30</td>
<td></td>
</tr>
<tr>
<td>July 5</td>
<td></td>
</tr>
<tr>
<td>July 19</td>
<td></td>
</tr>
<tr>
<td>July 24</td>
<td></td>
</tr>
<tr>
<td>July 57</td>
<td></td>
</tr>
<tr>
<td>July 63</td>
<td></td>
</tr>
<tr>
<td>July 63</td>
<td></td>
</tr>
</tbody>
</table>

CAES- Spring Open House 2011

www.ct.gov/caes
Number of pesticides in pollen loads (2009)

\[ r = 0.841 \]
\[ P = 0.061 \]
Conclusions

- Methods used can detect low concentrations of pesticides in pollen
- Pollen samples are heterogeneous, causing an increase in variability of data
- Honey bees are exposed to pesticides while foraging for pollen
- Pesticide concentrations vary with hive, time, and location
- Pesticides are a contributing factor to the problems faced by honey bees
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Managed Pollinator CAP
Coordinated Agricultural Project

A National Research and Extension Initiative to Reverse Pollinator Decline