Sub-lethal effects of pesticide residues in brood comb on worker honey bees

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Why are colonies dying?
A puzzle of interactions

- Lack of flowers - poor nutrition
- Environmental Pesticides
- Viruses
- In-Hive Pesticides
- Gut pathogen: *Nosema*
- Mite parasites
Research objectives

**Study 1: Pesticide load survey**
- Which, how many, & what levels are pesticide residues found in brood comb?
- Correlation between failing colonies & pesticide load?

**Study 2: Brood effects & adult longevity**
- Development & survivability (egg to adult emergence)
- Adult lifespan

**Study 3: Susceptibility expt.**
- To other pests and pathogens

Translating pesticide jargon

<table>
<thead>
<tr>
<th>Pesticide class</th>
<th>Active ingredient</th>
<th>Brand name</th>
</tr>
</thead>
<tbody>
<tr>
<td>pyrethroid</td>
<td>Tau-fluvalinate</td>
<td>Apistan, Hivestan</td>
</tr>
<tr>
<td>organophosphate</td>
<td>Coumaphos</td>
<td>Checkmite+</td>
</tr>
<tr>
<td>formamidine</td>
<td>Amitraz</td>
<td>Apivar, Tactic</td>
</tr>
</tbody>
</table>
Study 1: Pesticide load survey

Source of “dirty combs”

5 categories of samples ($n_{total}=98$):
1) PNW migratory colonies (poor) ($n=24$)
2) CRC (collaborative research colonies) ($n=24$)
3) QPO queen-producers ($n=19$)
4) FWS commercial foundation wax ($n=7$)
5) CCD suspect “CCD” (Dr. Jeff Pettis USDA, MD) ($n=24$)

Chemical analysis:

Samples sent to Roger Simonds at the USDA-AMS lab in Raleigh N.C.
171 most commonly used pesticides tested (including coumaphos, fluvinate, & amitraz metabolite (DMPF)).
Residues in combs determined with GC/MS.

What did we find?

Out of 171 screened, we found 66 different pesticides, 10 metabolites, and 1 adjuvant (piperonyl butoxide)

<table>
<thead>
<tr>
<th>% of pesticides detected</th>
<th>PNW poor=24</th>
<th>CRC=24</th>
<th>QPO Queen=13</th>
<th>Foundation=7</th>
<th>CCD=24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolites</td>
<td>19%</td>
<td>17%</td>
<td>26%</td>
<td>9%</td>
<td>17%</td>
</tr>
<tr>
<td>Insect/Acaricide</td>
<td>51%</td>
<td>67%</td>
<td>50%</td>
<td>68%</td>
<td>58%</td>
</tr>
<tr>
<td>Herbicide</td>
<td>9%</td>
<td>6%</td>
<td>7%</td>
<td>9%</td>
<td>2%</td>
</tr>
<tr>
<td>Fungicide</td>
<td>21%</td>
<td>10%</td>
<td>16%</td>
<td>13%</td>
<td>23%</td>
</tr>
<tr>
<td>Adjuvants</td>
<td>0%</td>
<td>0%</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Of the 47 insect/acaricides, 36 (77%) are toxic to bees!!!
Fungicides:
- detected in all categories
- highest in foundation wax

Metabolites:
- break down products
- coumaphos oxon is a major metabolite found in each category

Commercial Foundation Wax

- Recycled cell caps (used to store honey in comb)
- Used by beekeepers to draw new comb
- Wax caps removed (during honey extraction) & processed by boiling
- Boiling comb: no effect on residue levels in contaminated comb
- Heated, melted, & processed comb with known amounts of pesticides revealed residue levels 1.7x higher after processing

(Bogdanov et al. 1996)
Commercial Foundation Wax

High levels of 7 different fungicides in FWS, support prevalence of pesticides brought back by forager bees.

In addition, 21 insecticides, 4 herbicides & 4 metabolites were also detected.

Fungicides may affect bees

Direct effects:
- Increased mortality & growth abnormalities in larvae when fed fungicides
- Delayed effects – (captan), after 7 days toxicity of captan was 3-4x more toxic than assessment 72hrs after the same single dose exposure
  \[\text{(Mussen et al. 2004)}\]

Indirect effects:
- Synergistic effects (10-100x) (honey bees) when EBI fungicides are combined with pyrethroids
  \[\text{(Pilling and Jepson 1993)}\]
  EBI fungicides/neonicotinoids
  non EBI fungicides/neonicotinoids (shorter period)
  \[\text{(Schmuck et al. 2003)}\]
- Reduced repellency of pyrethroid/fungicide mixtures
  \[\text{(Thompson and Wilkins 2003)}\]
What insecticides did we find?

In-hive miticides account for majority of the insecticide load

*After one year, 50% of CRC were dead-outs!

**Average fluvalinate, coumaphos, & insecticide levels**

<table>
<thead>
<tr>
<th>Insect/Acaricide</th>
<th>PNW poor</th>
<th>CRC=24</th>
<th>Queen=13</th>
<th>Foundation=7</th>
<th>&quot;CCD&quot;=24</th>
</tr>
</thead>
<tbody>
<tr>
<td>fluvalinate</td>
<td>13086</td>
<td>17907</td>
<td>3565</td>
<td>0</td>
<td>4428</td>
</tr>
<tr>
<td>coumaphos</td>
<td>4970</td>
<td>1407</td>
<td>1133</td>
<td>1243</td>
<td>29730</td>
</tr>
<tr>
<td>Insect/Acaricide</td>
<td>18130</td>
<td>19479</td>
<td>5475</td>
<td>4880</td>
<td>34172</td>
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</tbody>
</table>

**Fluvalinate**

<table>
<thead>
<tr>
<th>Insect</th>
<th>PNW poor</th>
<th>CRC</th>
<th>Queen</th>
<th>Foundation</th>
<th>&quot;CCD&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>4214</td>
<td>17907</td>
<td>3565</td>
<td>3357</td>
<td>4428</td>
</tr>
<tr>
<td>range</td>
<td>164-12000</td>
<td>4010-92600</td>
<td>127-16400</td>
<td>236-12500</td>
<td>646-21300</td>
</tr>
</tbody>
</table>

Sub-lethal effects of fluvalinate in wax on queen rearing:

Significant reduction of queen weight at fluvalinate level of 3,550 ppb.

83 (97%) samples had fluvalinate and 56 (67%) had fluvalinate levels that exceed 3,550 ppb!

Note: the tolerance level of fluvalinate in honey is 50ppb!
**Coumaphos**

<table>
<thead>
<tr>
<th>coumaphos</th>
<th>PNW poor</th>
<th>CRC</th>
<th>Queen</th>
<th>Foundation</th>
<th>“CCD”</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>4190</td>
<td>1407</td>
<td>1133</td>
<td>1243</td>
<td>29730</td>
</tr>
<tr>
<td>range</td>
<td>205-22100</td>
<td>733-4130</td>
<td>0-4440</td>
<td>63.5-3140</td>
<td>2290-226000</td>
</tr>
</tbody>
</table>

**Sub-lethal effects of coumaphos in wax on queen rearing:**

50% rejection of queen cells at coumaphos level of 100,000ppb. (Pettis, J.S. et al. 2004. *Apidologie* 35: 605-610)


**Coumaphos was present in 85 (98.8%) of samples!**

Note: the tolerance level of coumaphos in honey is 100ppb!

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**Contamination in comb**

Residues from “in-hive” and environmental pesticides remain and accumulate in the comb for years


- 60% of comb had at least one systemic pesticide
- over 47% had fluvalinate, coumaphos, & chlorothalonil

![Entombed cells](image)
In-hive pesticide concerns

- Additive effect: two substances in combination produce a total effect the same as the sum of the individual effects (1+1=2)
  - *Ex: similar pesticides, multiple applications*

- Synergistic effect: the interaction of multiple compounds so that the sum of the combined effect is greater than either individual effect. (1+1>2)
  - *Ex: Apistan (pyrethroid) + Checkmite (organophosphate)*

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**Synergy of in-hive treatments**


<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>x-fold synergy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 µg coumaphos</td>
<td>2.1x (↑ flualinate toxicity)</td>
</tr>
<tr>
<td>3.0 µg coumaphos</td>
<td>4.4x (↑ flualinate toxicity)</td>
</tr>
<tr>
<td>10.0 µg coumaphos</td>
<td>32.1x (↑ flualinate toxicity)</td>
</tr>
<tr>
<td>1-3 µg fluvalinate</td>
<td>3.4x (↑ coumaphos toxicity)</td>
</tr>
</tbody>
</table>

**Synergy with environmental pesticides:**
- pyrethroid & piperonyl butoxide (PBO)= 980X;
- ergosterol biosynthesis inhibitors (EBI) fungicides & insecticides =10-100X;
Average insecticide level was significantly higher in brood comb from dead colonies (p=0.034).

Brood comb from dead colonies had 2x the amount of insecticide residues than from live colonies.

Concurrent detections of miticides

34% of brood comb from dead colonies had 3,500 ppb of each compared to 3% of brood comb from live colonies.
Graphs are not suggesting Varroa treatments killed colonies

Only that there may be a connection between pesticide load and the health of colonies

There’s more to the story…

Study 2: Brood effects study


Controls – newly drawn comb (coumaphos 21 ppb) or old comb from feral colonies with no detectable residues.

Treatments – (13 brood combs from previous survey)
- 24 insecticides, 9 fungicides, 3 herbicides, and 5 metabolites = 41 total

- # of pesticides ranged from 4 to 17, averaging 10

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Family</th>
<th>Average (ng/g)</th>
<th>Minimum (ng/g)</th>
<th>Maximum (ng/g)</th>
<th>LOD (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluvalinate</td>
<td>PYR</td>
<td>6,712</td>
<td>164</td>
<td>24,340</td>
<td>1</td>
</tr>
<tr>
<td>Coumaphos</td>
<td>OP</td>
<td>8,079</td>
<td>281</td>
<td>22,100</td>
<td>1</td>
</tr>
<tr>
<td>Coumaphos oxon</td>
<td>metabolite</td>
<td>596</td>
<td>10</td>
<td>1,850</td>
<td>5</td>
</tr>
</tbody>
</table>
Study 2: Brood effects & longevity

Enclosed queen laid eggs for 24 hrs on either block (up to 1000 eggs daily)

Brood effects

After 24 hrs. development of brood recorded at:

- day 1 (egg)
- day 4 (1st instar)
- day 8 (5th instar)
- day 12 (capped pupae)
- day 19 (incubation)

(Capped brood incubated (34°C))

Newly emerged adults

1) cage longevity tests
2) susceptibility expt
### Control

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coumaphos</td>
<td>20.9</td>
</tr>
</tbody>
</table>

### Test

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Hydroxycarbofuran</td>
<td>23</td>
</tr>
<tr>
<td>Aldicarb</td>
<td>20</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>32</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>4</td>
</tr>
<tr>
<td>Clothianidin</td>
<td>35</td>
</tr>
<tr>
<td>Coumaphos</td>
<td>22100</td>
</tr>
<tr>
<td>Coumaphos Oxon</td>
<td>1850</td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>7.9</td>
</tr>
<tr>
<td>Dinotefuran</td>
<td>97</td>
</tr>
<tr>
<td>Dithionylamine</td>
<td>281</td>
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<tr>
<td>Endosulphan 1</td>
<td>1</td>
</tr>
<tr>
<td>Fluvalinate</td>
<td>164</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>45</td>
</tr>
<tr>
<td>Malathion Oxon</td>
<td>22</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>22</td>
</tr>
<tr>
<td>Thiacloprid</td>
<td>113</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>38</td>
</tr>
</tbody>
</table>

#### Pesticide Combinations

- 2 Fungicides
- 3 Carbamates
- 1 OP + 2 metabolites
- 1 Cyclodiene
- 2 Pyrethroids
- 5 Neonicotinoids

**Total = 17**

#### Toxicity

- Highly toxic
- Moderately toxic

### Treatment comb: day 8

- 46% of treatment larvae expressed delayed development
Delayed larval development

Worker bees from another 3 treatment combs (sampled from CCD colonies) had similar effects.

An average of 19% of eggs remained unhatched at day 4

60-80% of those eggs were removed by day 8

Spotty brood pattern: reduces brood production efficiency

(queens must seek out empty cells, nurse bees must scatter around to care for brood)

(Mackensen 1950)

Adult worker emergence

Typical worker honey bee emergence occurs at day 20-21 of development.
Brood combs used multiple times

Delayed emergence?  
Effect on the colony?

Understanding *Varroa destructor*:

External parasitic mite  
Feeds on bee “blood”  
Circulates bee viruses  
Reason for *in-hive pesticides*
Varroa development

Pregnant mites leaves cell with adult bee; male & immatures die in cell

<table>
<thead>
<tr>
<th>DAYS SINCE CELLS CAPPED</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
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<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0%</td>
<td>13%</td>
<td>38%</td>
<td>94%</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

The mite family is completed by 15th day of bee development:

- mother mite & 4 progeny: (1st son, 1st daughter, 2nd daughter, 3rd daughter (egg))
- 3rd daughter only has a 13% chance of reaching adulthood.

http://www.ars.usda.gov/services/docs.htm?docid=2744&page=14

Varroa fecundity in drone brood

<table>
<thead>
<tr>
<th>DAYS SINCE CELLS CAPPED</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
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<th>15</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0%</td>
<td>13%</td>
<td>38%</td>
<td>94%</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

http://www.ars.usda.gov/services/docs.htm?docid=2744&page=14
**Varroa preference for drone brood**

Development time for drones is 23-24 days

Extra 2.5 days provides time for an additional 1-2 daughter mites to mature...the average # of daughters per mite in drone cells is about 2-2.5

Average # of daughters per mite in worker cells is 1.4-1.5

Average # of daughters per mite in worker cells with bees expressing delayed emergence?

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**Altered physiology of mite infested worker bees**

"Compared with non-infested workers, adult bees infested as pupae do not fully develop physiological features typical of long-lived wintering bees."

- reduced body weight, hemolymph volume, & abdominal carbohydrates
- reduced levels of key proteins (vitellogenin) for overwintering

**Late-autumn treatments do not protect developing winter bees from mites!!!!**

"Factor in colony loss during overwintering?"
Cage longevity

Newly emerged adults were counted, color-tagged

Cages were kept in incubators (30°C) without light

Mortality recorded daily

Gravity feeders: 50:50 sugar solution & water

Cage longevity results

Adult control bees outlived adult treatment bees in the cage by 4 days (p<0.005)

Average adult longevity in caged bees
**Study 3: Susceptibility experiments**


Examine possible susceptibility *Nosema* infection due to developmental exposure to pesticide residues

Collaboration with Matthew Smart
Colony 295:
formic acid acaricide, 
no Nosema inoculant

(low Varroa, 
normal Nosema)

Colony 103:
no acaricide, 
Nosema inoculant

(high Varroa, 
high Nosema)

- painted newly emerged worker bees (control & treatment) 
  and put into colonies 295 & 103.

- sample 20 bees from each treatment and colony: 
  (week 2, 3, 4 post-release)

- control & treatment bees tested for Nosema levels.

- no difference in Varroa mite levels between treatments.

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Purpose</th>
<th>Chemical family</th>
<th>Toxicity to bees</th>
<th>Detection (ng/g)</th>
<th>LOD (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comb Y</td>
<td></td>
<td></td>
<td></td>
<td>Comb Y</td>
<td></td>
</tr>
<tr>
<td>2,4 Dimethylphenyl formamide (DMPF)</td>
<td>Metabolite</td>
<td>Amidine</td>
<td></td>
<td>142</td>
<td>4</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>INSECT</td>
<td>OP</td>
<td>High</td>
<td>8.5</td>
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<tr>
<td>Coumaphos</td>
<td>INSECT</td>
<td>OP</td>
<td>Moderate</td>
<td>7230</td>
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<tr>
<td>Coumaphos oxon</td>
<td>Metabolite</td>
<td>OP</td>
<td></td>
<td>231</td>
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<tr>
<td>Endosulfan I</td>
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<td>OC</td>
<td>Mod</td>
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<td>1</td>
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<td>Mod</td>
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<td>INSECT</td>
<td>PYR</td>
<td>High</td>
<td>6800</td>
<td>1</td>
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<tr>
<td>Phosalone</td>
<td>INSECT</td>
<td>OP</td>
<td>Mod</td>
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<td>1</td>
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<td>THPI</td>
<td>Metabolite</td>
<td>Thiophthalimide</td>
<td></td>
<td>98.7</td>
<td>50</td>
</tr>
<tr>
<td>Comb G</td>
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<td></td>
<td></td>
<td>Comb G</td>
<td></td>
</tr>
<tr>
<td>2,4 Dimethylphenyl formamide (DMPF)</td>
<td>Metabolite</td>
<td>Amidine</td>
<td></td>
<td>147</td>
<td>4</td>
</tr>
<tr>
<td>Coumaphos</td>
<td>INSECT</td>
<td>OP</td>
<td>Mod</td>
<td>281</td>
<td>1</td>
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<tr>
<td>Coumaphos oxon</td>
<td>Metabolite</td>
<td>OP</td>
<td></td>
<td>10.2</td>
<td>5</td>
</tr>
<tr>
<td>Chlothrin</td>
<td>FUNG</td>
<td>Chloronitrile</td>
<td></td>
<td>65.7</td>
<td>1</td>
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<td>Fluvalinate</td>
<td>INSECT</td>
<td>PYR</td>
<td>High</td>
<td>11280</td>
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<tr>
<td>Permethrin total</td>
<td>INSECT</td>
<td>PYR</td>
<td>High</td>
<td>103</td>
<td>10</td>
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<tr>
<td>Pyrethrins</td>
<td>INSECT</td>
<td>PYR</td>
<td>High</td>
<td>229</td>
<td>50</td>
</tr>
</tbody>
</table>

Characteristics of pesticide residues detected in treatment combs Y & G: 
(OC, organochlorine; OP, organophosphate; PYR, pyrethroid; Mod, moderately toxic)
Effect of *Nosema* inoculants on spore level

<table>
<thead>
<tr>
<th>Mean spore level</th>
<th>Colony 295</th>
<th>Colony 103 (added Nosema)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G)</td>
<td>1,136</td>
<td>62,281</td>
</tr>
<tr>
<td>Treatment (comb G)</td>
<td>51,250</td>
<td>3,164,130</td>
</tr>
<tr>
<td>Control (Y)</td>
<td>3,488</td>
<td>170,212</td>
</tr>
<tr>
<td>Treatment (comb Y)</td>
<td>41,000</td>
<td>668,333</td>
</tr>
</tbody>
</table>

Colonies with Nosema inoculant added exhibited higher Nosema levels as expected.

*Nosema* susceptibility

Bees individually analyzed for *Nosema ceranae* spores (n=473)

Overall proportion of infected treatment bees was higher in both colonies.

There’s no difference in proportion of infected bees between colonies! (even with 50 mil spores added each week to colony 103)
**Nosema ceranae susceptibility**

Proportion of infected was higher all weeks for “dirty” comb Y & G (infection occurred in younger bees!)

Proportion infected & mean spore count increased over time for bees reared in “dirty” combs Y & G

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**Summary of findings**

**Pesticide load survey** - 75 different pesticides in brood comb (high of 22 in a single comb)

**Brood effects** – higher larval mortality, evidence for delayed development and adult emergence

**Adult longevity** – reduced by 4 days

**Susceptibility tests** - increased susceptibility to *Nosema*; higher proportion of infected bees & infection at younger age
Final thoughts

**Pesticide load survey** - synergistic interactions likely; chronic/long term effects unclear, effects from metabolites & fungicides unclear

**Brood effects** – less efficient brood production and care, over-worked queens, reproductive advantage for *Varroa* mites

**Adult longevity** – shortened lifespan increases colony need for brood production, pre-mature shifts in hive roles

**Susceptibility tests** - *Nosema* infection associated with immuno-suppressed bees? Stress related

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Use Varroa treatments that do not accumulate in the hive

<table>
<thead>
<tr>
<th>Pesticide class</th>
<th>Active ingredient</th>
<th>Brand name</th>
</tr>
</thead>
<tbody>
<tr>
<td>pyrethroid</td>
<td>Tau-fluvinate</td>
<td>Apistan, Hivestan</td>
</tr>
<tr>
<td>organophosphate</td>
<td>Coumaphos</td>
<td>Checkmite+</td>
</tr>
<tr>
<td>formamidines</td>
<td>Amitraz</td>
<td>Apivar, Tactic</td>
</tr>
<tr>
<td>essential oil</td>
<td>Thymol</td>
<td>Apiguard</td>
</tr>
<tr>
<td>organic acid</td>
<td>Formic acid</td>
<td>Miteaway</td>
</tr>
<tr>
<td>organic acid</td>
<td>Hop beta acids</td>
<td>Hopguard</td>
</tr>
<tr>
<td>organic acid</td>
<td>Oxalic acid</td>
<td></td>
</tr>
<tr>
<td>Grease patties</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food grade mineral oil mixtures</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hard treatments: synthetic or inorganic compounds

Soft treatments: natural or organic compounds (can still be toxic to bees)
## Comb replacement

### Benefits
- “diluting effect”
- ↓ # nosema spores, AFB spores, etc.
- ↓ risk of mite resistance
- ↓ synergy (coumaphos and fluvalinate)
- Improve efficacy of future treatments

### Disadvantages
- costly
- usually do not see immediate results
- need for replacement varies
- “diluting effect” will not be pesticide-free

## Signs to look out for

- Old dark thick comb
- Mark frames when brood diseases have been observed
- Multiple entombed pollen cells
- Scratch & sniff tests
Current research at UNL

- Integrated Pesticide Management for beekeepers
- Impacts of pesticide residues on Varroa fecundity
- Monitoring for abnormal bee losses (dead bee traps)
- Reporting bee incidents (improvements to reporting)
- Comb replacement recommendations
- Deactivation of pesticide residues in comb

Monitor for pesticide incidents
Dead bee traps

Easy to construct
Use to monitor abnormal loss of bees
Diagnose bees in traps

Reporting Bee Incidents

- State Agencies called to investigate
- High mortality in short period
- Samples in-hive & plants, soil, water
- Rule out other pests & pathogens

Problems:
- Mistrust/poor history
- Dwindling population (spiral effect)
- Unaware for weeks after initial event
- Too late for reliable residue sampling
- Weakened colonies have pests & pathogens
**Reporting Bee Incidents**

**Need:**
- Work with regulatory agencies
- To redefine examination criteria
  - Lethal → sub-lethal measures
  - Direct effects → indirect effects
- Improve metrics that can inform beekeepers of problem at start (rather than end of colony decline)
- Improve record keeping for colonies
- Public pesticide usage records/map
  - Used to estimate exposure & risk
  - Can help identify potential toxins for pesticide testing

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This study illustrates that pesticides can have *subtle, sub-lethal, & indirect* effects that can have serious colony level consequences.

**Take home message:**
- Regular comb replacement
- Monitor mite levels
- Use softer organic acid
- Appropriately timed treatments
- Monitor for bee losses
- Record information: (date, colony, health status, mite level, treatments)

**QUESTIONS ???**
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