Environmental Toxicology of Pesticide Residues to Pollinators

Virginia Tech Activities to Further Understand Bee Health Concerns

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Pollinator Declines: A Global Problem

- Global ecosystem services and agricultural production threatened due to insect pollinator declines.
- Bees contribute ~ 80% of insect pollination.
- Economic value of managed bee pollination is ~ $14 bill. in the United States and ~ $220 bill. worldwide.
- Causal explanation for bee population decline is unclear, despite active research efforts.

Without bees they’ll all be off the menu
Bee Colony Decline: A Virginia Problem

- Bee colony losses ca. 30% across the Commonwealth of Virginia;
- Crop pollination fees, honey sales, and colony replacement costs in Virginia ($1.3 to 1.8 mill.);
- Bee colonies for crop pollination are essential to beekeepers and growers to sustain the food and fiber needs of our society.
Bee Colony Decline: Multiple Stressors

Multiple stressor interactions associated with bee colony failure. Blue boxes represent the three main groups of stressors associated with bee loss; red arrows represent direct pressures on bees from stressors; green arrows represent interactions between stressors; and blue arrows represent interactions within stressors. (Modified from Potts et al. 2010, Trends in Ecol. and Evol. 25(6):345-353)
Pesticide Exposures and Bee Health

- Multiple agrochemicals and miticides in North American bee hives;
- Major pesticide classes:
  - PYR (99%)
  - OP (99%)
  - FUNG (60%)
  - HERB (50%)
  - CB (20%)
  - NEO (1-3%)
- Mixtures of agrochemicals present in ca. 92% of bee, wax, and pollen matrices;
- Acute toxicity DOES NOT equal ecological health risk.
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Sub-Lethal Effects of Miticide Exposures

- Miticide residues in brood comb (Wu et al. 2011):
  Development and emergence
  Adult longevity
  Hive labor and foraging behaviors
  Immunodeficiencies

- Virginia Tech Apiculture Program (Fell and Tignor 2001; Burley et al. 2008):
  Impaired reproductive physiology
  Reduced queen rearing
  Reduced sperm viability
  Increased queen failure and loss

Dr. Rick Fell and Jackson Means
Hive Antibiotic Alters Miticide Toxicity

Data by Jennifer Williams

**tau-Fluvalinate + Antibiotic**

24-h LC$_{25}$ = 0.50 ng/µl

- Bee mortality increased ~ 50% in *tau*-fluvalinate-treated bees
- P450 activity decreased ~ 45% in oxytetracycline-treated bees

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VirginiaTech
Invent the Future
Hive Antibiotic Alters Miticide Toxicity

Bee mortality decreased ~ 80% in coumaphos oxon-treated bees

Esterase activity increased ~ 20% in oxytetracycline-treated bees

Data by Jennifer Williams

Coumaphos oxon + Antibiotic

24-h LC$_{25}$ = 0.15 ng/µl

-蜂死亡率降低约80%在苯菌硫磷氧化物处理的蜜蜂
-酯酶活性增加约20%在四环素处理的蜜蜂

24-h LC$_{25}$ = 0.15 ng/µl

Diethylphosphoric Acid

Esterase Hydrolysis

Cloroferron

Esterase Hydrolysis

Coumaphos (Organophosphate)

Coumaphos Oxon

P450 Desulfuration

Crop Fungicide Alters Miticide Toxicity

Data by Jennifer Williams

Bee mortality decreased ~ 50% in *tau*-fluvalinate-treated bees, and increased ~ 35% in coumaphos oxon-treated bees.

P450 activity reduced ~ 50% and esterase activity increased ~ 20% in chlorothalonil-treated bees.
Current Activities for Bee Health Concerns

- **Goal:** Provide a comprehensive examination of bee health in VA.

- **Question:** To what extent are bee health profiles related to pesticide exposures?

- **Specific aims:**
  - Examine the nutrition and immune status of pesticide-treated bees
  - Examine the presence and prevalence of pathogens in pesticide-treated bees
  - Examine the epidemiological patterns of pesticide exposures and bee health

- **Deliverables:** Develop bee health thresholds to improve management practices for the beekeepers, growers, and pesticide applicators.
Field Study of Pesticide-Treated Hives

- Natural hives established at Price’s Fork, Moore Farm, and Kentland Farm apiaries (Blacksburg, VA);
- Hives treated with miticide-impregnated strips (i.e., manufacturer label recommendations) or fungicide solution and maintained for six weeks.
Symbiome Structure and Bee Health

- Bees process plant nectar to honey, a carbohydrate source for the colony, and plant pollen provides individuals with amino acids, lipids, vitamins, and minerals.

- Symbiome is a distinctive microbial community that regulates the nutrition and immune status bees, and can be negatively impacted by pesticide exposures (Tian et al. 2012).

- Nutrition deficiencies, or stress, can reduce immunocompetence and increase pathogen susceptibility of individual bees resulting in colony failure.
Illumina sequencing of 16SrRNA and ITS genes for bacteria and fungi;

Bacteria: ca. 74,690 sequences and 517 OTUs at 3% evolutionary distance;

*Lactobacillus* is reduced ca. 50% in *tau*-flualvalinate and chlorothalonil-treated bees;

Bee health concern: *Lactobacillus* sps. are beneficial symbionts for carbohydrate metabolism, immunocompetence, and pathogen defense.

Phyla: Proteobacteria (45%), Firmicutes (36%), Actinobacteria (18%), Cyanobacteria (1%), and Bacteroidetes (0.2%)

Genera: *Lactobacillus, Bifidobacterium, Edwardsiella, Serratia*, and *Bartonella* (84%)
Illumina sequencing of 16SrRNA and ITS genes for bacteria and fungi;

Bacteria: ca. 74,690 sequences and 517 OTUs at 3% evolutionary distance;

*Edwardsiella* is reduced ca. 30% in coumaphos-treated bees, and *Bartonella* is increased ca. 90% in tau-fluvalinate and chlorothalonil-treated bees;

Bee health concern: *Edwardsiella* is important for carbohydrate and nitrogen metabolism, but *Bartonella* is an opportunistic pathogen.

Phyla: Proteobacteria (45%), Firmicutes (36%), Actinobacteria (18%), Cyanobacteria (1%), and Bacteroidetes (0.2%)

Genera: *Lactobacillus, Bifidobacterium, Edwardsiella, Serratia, and Bartonella* (84%)

Data by Dr. Madhavi Kakumanu and Alison Reeves
Illumina sequencing of 16SrRNA and ITS genes for bacteria and fungi;

- Fungi: ca. 19,080 sequences and 373 OTUs at 3% evolutionary distance;
- Phyla: Ascomycota (72%), Basidiomycota (21%), Glomeromycota (7%), and Unspecified (0.1%);
- Genera: *Penicillium*, *Aspergillus*, *Cladosporium*, and *Alternaria* (molds) in addition to *Saccharomycetes*, *Torulopsis*, and *Candida* (yeasts) are present, but numbers are highly variable between pesticide treatments;

- ca. 20% of fungal sequences are *Alternaria*, *Cladosporium*, and *Metschnikowia* sps. (i.e., major role in nectar to honey production);

**Bee health concern:** Pesticides may impact fungal community structure; however, the negative effects of coumaphos exposure may limit carbohydrate production and lead to nutritional stress at the individual and colony level.
Pesticides Reduce Bee Nutritional Status

Data by Alison Reeves

![Graph showing comparison of proteins, carbohydrates, and lipids in bees exposed to different pesticides.](image)

- **Proteins (µg/ml)**
  - Pre-Exposure Baseline: 1507.01 ± 61.92 µg/ml
  - Significant reduction in tau-fluvalinate and chlorothalonil-treated bees relative to control.

- **Carbohydrates (µg/ml)**
  - Pre-Exposure Baseline: 572.50 ± 25.74 µg/ml
  - Significant reduction in tau-fluvalinate and chlorothalonil-treated bees relative to control.

- **Lipids (µg/ml)**
  - Pre-Exposure Baseline: 449.95 ± 13.96 µg/ml
  - Significant reduction similar to proteins and carbohydrates.

**Bee health concern:** Pesticides may impact the bee symbiome resulting in nutritional stress and impaired bee health.
Phenoloxidase activity is increased ca. 70% in pesticide-treated bees relative to control.

Bee health concern: Pesticides may elicit nutritional stress (e.g., protein deficiency) and reduce immunocompetence leading to increased pathogen infection at the individual level (i.e., phenoloxidase stimulation).

**Phenoloxidase activity is a parameter of individual immunity expressed in the hemolymph of bees.** POX is a cellular and humoral response that catalyzes the encapsulation of pathogens to provide immune protection to individual bees.
Glucose oxidase activity is increased ca. 35% in pesticide-treated bees relative to control.

Bee health concern: Pesticides may elicit nutritional stress (e.g., protein deficiency) and reduce immunocompetence leading to increased pathogen infection at the colony level (i.e., glucose oxidase stimulation).
Pesticides Increase Bee Pathogen Infection

- *Nosema ceranae* is an obligate, intracellular fungal pathogen;

- *Nosema* infection is predominant in worker bees, but can affect both drones and queens (Traver and Fell 2011, 2012);

- *Nosema* infection is significantly higher in coumaphos- and chlorothalonil-treated bees.

- Bee health concern: Nutrition and immune deficiencies of pesticide-treated bees may increase pathogen susceptibility (e.g., viral, bacterial, or fungal).

**Detection of *Nosema ceranae* infection in pesticide-treated bees.** Pathogen levels are on the y-axis reported as the average copy number transformed using log(average copy number + 1). On the x-axis are the treatments administered. For each treatment, *N. ceranae* levels are given for pre-treatment (open circles) and six weeks post-treatment (filled circles). The average *N. ceranae* level for each time point and treatment is shown with the red asterisk.
Population Dynamics Model for Bee Health

- Predictive model to explore the impact of bee health on colony growth and development;
- Calculate critical threshold for which colonies regulate a stable population size;
- Provide a theoretical framework for experimental studies to explain bee health thresholds and colony failures.

Quantitative Model of Bee Colony Population Dynamics (Khoury et al. 2011, PLoS ONE 6(4):e18491)
Research Summary and Long-Term Goals

- Bee decline is a nationally-recognized problem, demanding attention from the general public, scientific community, and beekeeping industry.

- To what extent are bee health profiles related to pesticide exposures?

- Research collaborations for “pesticides and sustainable pollination services of wild and managed bees”: Virginia Department of Agriculture and Consumer Services, Southern Illinois University, University of Maine, and University of Exeter.

- Teaching and outreach activities: Post-doctoral and student training programs, beekeeper workshops and pollinator conferences, reference guides for beekeepers and pesticide applicators, and University events.

- Long-term goal: Provide real world, science-based solutions to address bee health concerns and translated to management practices to improve pollinator health and maintain a viable apiculture industry in Virginia, and the United States.
Real-world problems meet creative, science-based solutions...
Troy Anderson’s research group studies the effects of pesticides on honey bee hives.

Research Team: Rick Fell, Brenna Traver, Carlyle Brewster, Mike Lydy, Mark Williams, Alison Reeves, Jennifer Williams, Jackson Means, and Cameron Rose

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