The Pesticide Stewardship Alliance, 13th Annual Pesticide Stewardship Conference 5-7 February 2013, Mobile, Alabama



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.



News » Nation = Demographics = Troops at Risk = Lotteries

U.S. losing bees and beekeepers

Updated 4/9/2008 11:14 AM | Comments 🖳 8 | Recommend 🔂 8



Enlarge By Kalim A. Bhatti, USA TODAY

Beekeeper David Hackenberg works on his hive in Lewisburg, Pa., April 29, 2007. Hackenberg has lost nearly \$400,000 from the mysterious bee deaths across the country. By Heather Collura, Special for USA TODAY

The number of bees is on the decline across the USA, and there's also a shortage of beekeepers.

The number of commercial beekeepers is dwindling because the business of keeping bees is not as profitable as it once was, according to Jeff Pettis, research leader at the U.S. Department of Agriculture Bee Research Laboratory in Maryland.

That decline in profitability is due in large part, Pettis said, to lower honey prices — the average U.S. price per pound dropped fourtenths of a cent over the past year. Keepers also face difficulty in keeping healthy bees





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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High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health

Christopher A. Mullin¹*, Maryann Frazier¹, James L. Frazier¹, Sara Ashcraft¹, Roger Simonds², Dennis vanEngelsdorp³, Jeffery S. Pettis⁴

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tau-Fluvalinate (Miticide; Apistan®)



Chlorothalonil (Fungicide; Bravo®)



Coumaphos (Miticide; Checkmite+®)





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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

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PLos one

Sub-Lethal Effects of Pesticide Residues in Brood Comb on Worker Honey Bee (*Apis mellifera*) Development and Longevity

Judy Y. Wu, Carol M. Anelli, Walter S. Sheppard* Department of Entomology, Washington State University, Pullman, Washington, United States of America

APICULTURE AND SOCIAL INSECTS

Survival of Honey Bee (Hymenoptera: Apidae) Spermatozoa Incubated at Room Temperature from Drones Exposed to Miticides

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Dr. Rick Fell and Jackson Means



Hive Antibiotic Alters Miticide Toxicity



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





Crop Fungicide Alters Miticide Toxicity



- 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

- Question: To what extent are bee health profiles related to pesticide exposures?
- Goal: Provide a comprehensive examination of bee health in VA.
- > 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







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.



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Pesticides Impact Beneficial Bee Symbiome

- 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-fluvalinate and chlorothaloniltreated bees;
- Bee health concern: Lactobacillus sps. are beneficial symbionts for carbohydrate metabolism, immunocompetence, and pathogen defense.

Data by Dr. Madhavi Kakumanu and Alison Reeves



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

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



Pesticides Impact Beneficial Bee Symbiome

- 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 chlorothaloniltreated bees;
- Bee health concern: Edwardsiella is important for carbohydrate and nitrogen metabolism, but Bartonella is an opportunistic pathogen.

Data by Dr. Madhavi Kakumanu and Alison Reeves



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

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



Pesticides Impact Beneficial Bee Symbiome

- 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 Alterneria (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 Alterneria, 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



nvent the Future

Pesticides Impact Bee Individual Immunity





Data by Alison Reeves

Nature Reviews | Microbiology

Phenoloxidase activity is increased ca. 70% in pesticide-treated bees relative to control. **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.

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).



Pesticides Impact Bee Social Immunity





Glucose oxidase activity is increased ca. 35% in pesticide-treated bees relative to control. **Glucose oxidase activity is a parameter of social immunity expressed in the hypopharyngeal glands of bees.** GOX catalyzes glucose oxidation to gluconic acid and hydrogen peroxide, which has antiseptic properties and promotes disease prevention at the group level.

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).



Data by Alison Reeves

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 pesticidetreated 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.

www.fralin.vt.edu

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Virginia Department of Agriculture and Consumer Services

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