Fungicide Effects on Honey Bee Development

Project No.: **13-POLL12-Hooven**

Project Leader: Louisa A. Hooven, PhD
Department of Horticulture
4017 ALS
Oregon State University
Corvallis, OR 97331
Louisa.Hooven@OregonState.edu

Project Cooperators and Personnel:

Cooperators: Ramesh Sagili, Dept. of Horticulture, Oregon State University
Jim Adaskaveg, Dept. of Plant Pathology and Microbiology, UC Riverside
Eric Mussen, Dept. of Entomology, UC Davis
Oregon State University Statistics Consulting Service

Personnel: Undergraduate Students:
Cole Ditzler, Sarah Montague, Josean Perez, Matt Stratton, Ann Bernert, Joe Taylor, Montana Miller, Aron Montoya, Julia Lofesnes, Kristina Hauge, and Andrea Methven

Objectives:

Some beekeepers report problems with honey bee development during almond pollination [1], and suspect that one or more fungicides may be responsible. Although some fungicides have been shown to be toxic to honey bee larvae in laboratory tests [2] and may accumulate in hive materials [3-5] , it is unclear how closely those experiments resemble field exposures. In 2012 we performed preliminary semi-field experiments to determine whether levels of fungicides commonly applied during almond pollination affect honey bee development and colony health. In 2013 our overall goal was to validate our preliminary data suggesting iprodione (Rovral) may affect colony health.

Our objectives include:

- Determine appropriate experimental concentrations of iprodione
- Expose bees to 1X, 10X, and 10X formulated iprodione (Rovral), with 1X representing concentrations known to occur or calculated to occur in pollen
- Observe whether larval development is affected in treated colonies
- Observe whether overall colony health is affected in treated colonies.

In 2014, we are following through on this project. First, we are actively working to complete a research paper describing our results, and are working with the OSU Statistics Consulting Service to rigorously analyze our data for this purpose. We are also including work done in the Sagili lab examining effects of fungicides on growth of microorganisms found in bee gut. We are also assembling a review paper discussing the potential effects of some fungicides on pollinators.

Second, following the reports of problems with honey bees during the 2014 almond pollination cycle, we performed a similar field experiment exposing bees to Tourismo and its active ingredients. The reports of loss of a brood cycle did not sound dissimilar from previous reports that have inspired investigations of Pristine and other crop protection agents used during almond bloom. The Toursmo insecticide/insect growth regulator product was at one time tentatively linked to 2014 bee losses, although this correlation was far from conclusive. The effects of Tourismo were suspected by some to be exacerbated by fungicide co-application, and we included a fungicide in our work. We are only beginning to analyze our data from this project.

The Tourismo field experiment was performed under a USDA NIFA grant to examine whether nano-enabled pesticide particles cling to bees, and whether this exacerbates accumulation in the colony or residual toxicity. We are examining several products used in almonds in this work, including Tourismo and Rovral.

Interpretive Summary:

Beekeepers have reported brood loss several weeks after fungicide application. Similarly, in 2012 we observed decreased colony growth compared to controls several weeks after treatment with iprodione and chlorothalonil in pollen, as measured by weekly evaluations of eggs, larvae, and capped brood. To validate these findings, in 2013 we repeated these studies with a larger number of colonies, focusing on iprodione (Rovral). We made similar observations to 2012, and observed decreased colony growth compared to controls. However, due to the natural variation between bee colonies, we are finalizing work with a statistician in order to report our data in the most unbiased way possible.

Our data may explain beekeeper reports of delayed toxicity to brood, although many other fungicides and other products are used on and around almonds during bloom, and should be explored for potential effects on honey bees. Additionally, bees are exposed to pests, pathogens, and variations in weather, nutrition, and beekeeper practices during almond pollination.

We hope that our results will inspire beekeepers and almond growers to consider practices that further decrease honey bee exposure to fungicides, including the timing and placement of hives, and the timing of sprays. Honey bee colonies are exposed to fungicides during pollination of many crops, and future research should consider the additive and synergistic effects of these exposures.

Pesticide concentrations: Iprodione concentrations were determined from pollen collected in multiple orchards in 2011 and 2012 in the Turlock area during almond bloom. Samples were analyzed by USDA/ARS in North Carolina, and/or Environmental Micro Analysis (Woodland, CA) for multiple pesticides. These concentrations were compared to those discussed in the literature or in research presentations, concentrations extrapolated from other fungicide applications, and together were found to be similar to concentrations calculated by the EPA T-Rex method [6]. Using this approach, we calculated that a field concentration of iprodione in pollen collected by bees (1X) = 30 mg/g (rounded up from 27.5). For risk assessment purposes, we also used a 10X concentration of 300 mg/kg, and used this figure to calculate 10X formulated iprodione (Rovral). **Amending pollen with iprodione**: Pollen from a non-

agricultural area was obtained from Hummingbird Wholesale (Eugene, OR). The fungicides were dissolved in acetone, and whisked into the pollen. Rovral was used undiluted, and was difficult to mix into pollen. The pollen was laid on trays so that the acetone could better evaporate. The pollen was packed into fully drawn plastic honey comb (Permacomb) which had been dipped into pesticide-free wax. **Flight Cages:** 8 x 8' Flight cages were constructed, using Excel 30124 40 x 25 insect netting (US Global Resources), over PVC pipe. **Honey Bees:** Nucleus colonies were obtained from Queen Bee Honey Company, Corvallis, Oregon, and transferred to standard 10-frame hives before the experiment. **Exposure:** After initial evaluation (see below), 7 colonies each treatment were provided with a Permacomb frame packed with pollen treated with acetone only, acetone with 1X or 10X iprodione, or 10X Rovral. Flight cages were erected over the colonies, so that only the treated pollen was available to each colony for consumption. After 1 week, the flight cage was removed, and bees allowed to forage freely. A plastic frame feeder with cap/ladder was provisioned with 2:1 sugar syrup within the hive, and water was provided in a jar feeder within the flight cage.

Evaluations: Each colony was evaluated weekly, frame by frame for coverage of bees, pollen, nectar, honey, eggs, larvae, and capped brood. Areas of eggs and larvae were mapped in detail in order to follow development in each subsequent evaluation. The colonies were evaluated for a total of 6 weeks. To avoid bias, evaluators remained unaware of the treatment of each hive.

Colony Health: Bees were collected from each colony at the first evaluation, after treatment, and at the conclusion of the experiment. These samples will be examined for varroa and nosema levels, and for hypopharyngeal gland protein levels, per protocols in the Sagili laboratory.

Results and Discussion:

Similar to 2012 results, in colonies treated with 1X and 10X iprodione, we observed less development of capped brood than in controls beginning at 3 weeks from the initiation of treatment. This trend was also observed with larvae, but less clear with eggs. Total brood is illustrated in **Figure 1**. We also observed increased Nosema levels in colonies treated with iprodione. Effects of the formulated product, Rovral, are less clear, possibly due to the difficulty of homogeneously incorporating this material into pollen, creating uncertainty as to whether bees consumed it. We are currently working with a statistician to represent our 2011/2012 data accurately for publication. We can tentatively conclude that field exposures of iprodione may adversely affect honey bee

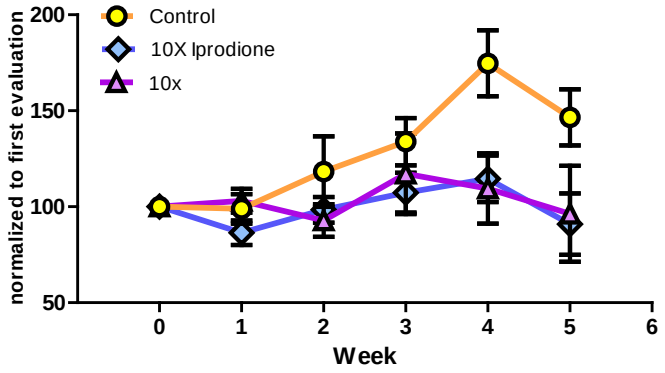

Figure 1. Effects of iprodione on brood. Total brood (eggs, larvae, and capped brood) is greater over time in controls compared to a field concentration (1X) of Iprodione, or 10 times field concentration (10X). Bees were treated at week 0.

development. As we write, we are examing how recent publications agree with our results [7, 8].

Initial analysis of Tourismo, Tourismo active ingredients, and Tourismo combined with the fungicide Protocol do not reveal any effect on brood compared to controls at the concentrations used, although we are examing other colony health measures from collected samples (**Figure 2**).

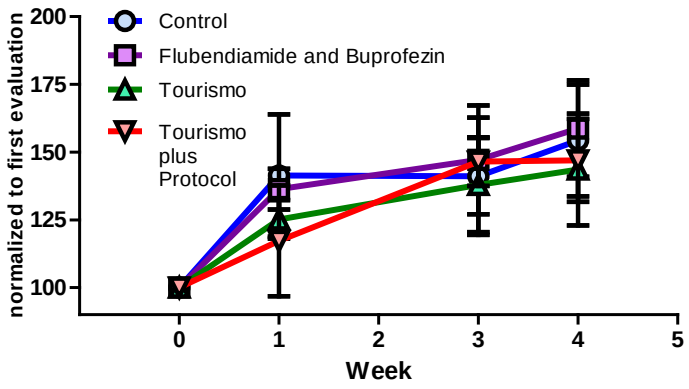

Figure 2. Preliminary analysis of colonies treated with Tourismo reveal no effect on brood. No difference was observed between control colonies and those treated with Tourismo, Tourismo active ingredients flubendiamide and buprofezin, or Tourismo and Protocol (a fungicide with propiconazole and thiophanate-methyl as active ingredients).

Fungicides are examined for potential effects on bees during the pesticide registration process. Only testing direct effects on adult bees, without considering larval development, is a drawback of these tests. However, there is a lack of any established mechanism of toxicity to bees that fungicides might act through. It is important to consider the biology of honey bees in detail to propose possible mechanisms. Generally, pesticides coming into the colony with pollen would go through a fermentation process, as raw pollen is transformed into bee bread. No one has yet investigated how the microbial communities involved in this process metabolize environmental contaminants. There have been some studies indicating that fungicides affect the growth of these microbes, although whether field concentrations cause this effect should be explored further.

Young adult honey bees consume the majority of pollen and bee bread. They then use the protein in the pollen to develop hypopharyngeal glands and create proteinaceous secretions (brood food and royal jelly) which are fed to developing larvae. Some fungicides, including iprodione, affect development in other species [9]. Larvae consume a very small amount of bee bread directly, compared to these secretions, rending investigations directly feeding fungicides to larvae difficult to interpret [10]. For larvae to directly consume a fungicide, it would need to persist through pollen fermentation, and through the metabolic system of nurse bees. If developmental toxicity of any fungicide is explored further, the route of transfer of the parent compound via pollen through fermentation to bee bread, and through nurse bee process will need to be tracked, in order to determine final extent of larval exposure. Additionally, as the nutrition of larvae is dependent on development of hypopharyngeal glands in nurse bees, this process should also be evaluated as a potential target of toxicity.

Research Effort Recent Publications:

We are working on two publications based directly on the Almond Board project. The first will report our research results, and include work by an undergraduate in Ramesh Sagili's lab, Ann Bernert, exploring the effects of fungicides on growth of honey bee gut microbes.

The second publication is a review discussing possible mechanisms of toxicity and unanswered questions about fungicides and honey bees. In addition to possible toxicity of some fungicides to bees, this will include a discussion of Pristine. Pristine was implicated in colony problems, but invesitgations by multiple researchers were unable to find significant toxicity to bees at field concentrations.

Future publications will describe our work with Tourismo, and nanoenabled pesticide formulations.

In addition to the presentation and poster at the 2013 Almond Board conference, presentations of our work were made at:

- America Bee Research Conference, San Antonio, Texas;
- Impacts of Pesticides on Honey Bee Health Conference, London, England;
- Effect of Fungicides on Development and Behavior of Honey Bees, Department of Horticulture, Oregon State University;
- Apimondia International Apicultural Congress, Kiev, Ukraine; and
- An abstract was sent to the Society of Toxicology Annual Meeting.

References Cited:

1. Mussen, E. *Fungicides Toxic to Bees?* Apiculture News, 2008. Nov/Dec.
2. Mussen, E.C., Peng, C.Y.S., and Lopez, J.E., *Effects of Selected Fungicides on Growth and Development of Larval Honey Bees, Apis mellifera L. (Hymenoptera: Apidae).* Journal of Environmental Entomology, 2004. 33(5): p. 1151-1154.
3. Mullin, C.A., et al., *High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health.* Plos One, 2010. 5(3): p. e9754.
4. Vanengelsdorp, D., et al., *"Entombed Pollen": A new condition in honey bee colonies associated with increased risk of colony mortality.* Journal of Invertebrate Pathology, 2009. 101(2): p. 147-149.
5. vanEngelsdorp, D., et al., *Colony Collapse Disorder: A Descriptive Study.* Plos One, 2009. 4(8).
6. Office of Chemical Safety and Pollution Prevention, O.o.P.P., Environmental Fate and Effects Division, USEPA; Environmental Assessment Directorate and H.C.C.D.o.P.R. Pest Management Regulatory Agency, *White Paper in Support of the Proposed Risk Assessment Process for Bees*, 2012.
7. Pettis, J.S., et al., *Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen Nosema ceranae.* Plos One, 2013. 8(7): p. e70182.

8. Yoder, J.A., et al., *Fungicide Contamination Reduces Beneficial Fungi in Bee Bread Based on an Area-Wide Field Study in Honey Bee, Apis mellifera, Colonies.* Journal of Toxicology and Environmental Health-Part a-Current Issues, 2013. 76(10): p. 587-600.
9. Blystone, C.R., et al., *Iprodione delays male rat pubertal development, reduces serum testosterone levels, and decreases ex vivo testicular testosterone production.* Toxicology Letters, 2007. 174(1-3): p. 74-81.
10. Zhu, W.Y., et al., *Four Common Pesticides, Their Mixtures and a Formulation Solvent in the Hive Environment Have High Oral Toxicity to Honey Bee Larvae.* Plos One, 2014. 9(1).