Fungicide Effects on Honey Bee Development

Project No.: **11-POLL12-Hooven**

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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], it is unclear how closely those experiments resemble field exposures. Our overall goal is to determine whether levels of fungicides commonly applied during almond pollination affect honey bee development in a semi-field setting. Our objectives include:

- Determine concentrations of fungicides that bees may be exposed to
- Expose bees to similar concentrations of fungicides
- Observe whether development of bees is affected in treated colonies
- Sample pollen to determine whether fungicides are degraded by the microorganisms in the colony during the experiment.

We chose several commonly used fungicides for this study, including iprodione (Rovral), ziram, chlorothalonil (Bravo, Echo), and boscalid/pyraclostrobin (Pristine) based on their frequent use in almonds, toxicity in laboratory tests [2], links to honey bee health problems reported in the literature [1, 3], and likelihood to persist in hive materials [4]

Interpretive Summary:

As of this report, we are two weeks into our experiment, and as yet have observed no obvious signs of toxicity in developing or adult bees. However, beekeepers have reported brood loss several weeks after fungicide application, and we may yet see effects as we continue to observe these colonies. We are working with an analytical chemist at OSU to track how long

these agrochemicals persist in pollen. When completed, this study is intended to identify which if any of these fungicides have a potential effect on bees and require future study.

Materials and Methods:

POLLEN: Collection: Pollen traps were placed on honey bee colonies in multiple orchards in 2011 and 2012 in the Turlock area during almond bloom. **Analysis:** Almond Pollen samples were analyzed by USDA/ARS in NC, and/or Environmental Micro Analysis (Woodland, CA) for multiple pesticides. **Concentrations for feeding bees:** Concentrations 10 times the greatest amount found in these results or in the literature [4] of iprodione, chlorothalonil, ziram, and boscalid plus pyraclostrobin were used to spike pollen, in order to represent a worst case scenario. Pollen from a non-agricultural area was obtained from Hummingbird Wholesale (Eugene, OR), and will be analyzed to verify low pesticide content. The fungicides were dissolved in 100 ml of acetone, and whisked into 2 kg of 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. **Ongoing Analysis:** Kim Anderson's laboratory (Dept. of Toxicology, Oregon State University), will be analyzing pollen throughout the remainder of the experiment.

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 Foothills Honey, Colton Oregon, and reduced to 4 frames.

EVALUATIONS: Each colony was evaluated 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 fungicide-spiked pollen frame was then added, and the bees were enclosed in the flight cage. 4 colonies each were treated with ziram, iprodione, chlorothalonil, and boscalid/pyraclostrobin. 4 colonies were treated with acetone only, and 2 were untreated. Water and 30% sugar solution were offered *ad lib*. After 7days, the flight cage was opened and the bees were allowed to forage freely. Each colony was again evaluated in detail, and will be evaluated weekly for several more weeks. To avoid bias, evaluators were kept unaware of the treatment of each hive. To track development, the data from each of these evaluations will be compared across time.

Results and Discussion:

Some beekeepers have reported an effect on honey bee larvae during or after almond pollination, and suspect fungicides [1]. It is important to note that not all beekeepers report such losses. This implies that there is a specific set of conditions which cause this loss, which could include a specific fungicide, adjuvant [5], synergy between agrochemicals [6], pest, disease, or colony management practice experienced by only certain beekeepers. As part of a larger proposed study of the health of honey bees in almonds, we are first asking whether fungicides commonly applied during almond bloom contribute to this phenomenon.

We first analyzed the levels of multiple pesticides, including fungicides in almond pollen in 2011 and 2012. Not surprisingly, the spectrum of chemicals found varies greatly between years and locations, and of course we cannot hope to represent the entire almond growing

region in our limited sample set. This provided us with a sense of the concentrations of some of the fungicides that bees may actually be exposed to during almond bloom. Insecticides, herbicides, and miticides such as amitraz, used by beekeepers to control varroa mites, are also found in our samples.

Fungicide Active Ingredient	Highest Field Levels Found	Used in Study
Chlorothalonil	18.77ppm [4]	200 ppm
Boscalid	3.36 ppm	35 ppm
Pyraclosrobin	1.48 ppm	10 ppm
Iprodione	5.84 ppm	60 ppm
Ziram	ND	200 ppm

To reflect a worst-case scenario, we treated pollen with 10 times the highest concentrations found (**Table Above**), and packed the pollen in a fully drawn wax-coated plastic frame. Covering the bees in a flight cage for a week forced them to consume at least some of the treated pollen.
We evaluated each colony before and after the pollen treatment, and are currently continuing with weekly evaluations. Although we have seen little if any signs of toxicity in the first two weeks, reports by beekeepers describe effects 17 days after fungicide treatment [1].

While fungicides are generally of low toxicity to adult bees [7], some bee researchers are currently exploring whether fungicides inhibit the normal fungi and yeast in bee hives from fermenting pollen into bee bread, which is reportedly necessary for bee nutrition [8]. While this could explain beekeeper reports of delayed toxicity, it is unclear whether the amounts of fungicides found in the field could have this effect. If a given fungicide affects the ability of nurse bees to provide nutrition to larvae, this should be reflected by effects on development observed in our colony evaluations.

Conversely, the bacteria and fungi that ferment pollen could degrade pesticides before they are consumed by bees. To test this, we are measuring the levels of pesticides in the pollen after it has been in the hive for two weeks. If we see no effects on brood in our colony evaluations, biodegradation of pesticides could provide assurance that long term effects from a given fungicide are unlikely. We expect that our study will indicate whether potential effects of Iprodione, Chlorothalonil, Boscalid/Pyraclostrobin, or Ziram on honey bees merit further investigation.

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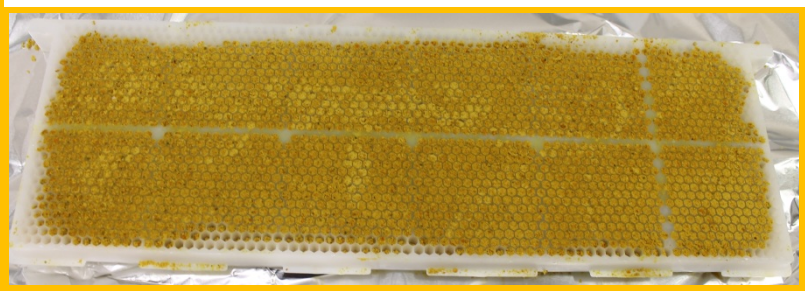

Bees were kept in a flight cage for 7 days and forced to consume fungicide-treated pollen (left inset). Before and during the experiment, the colonies are being evaluated in detail for brood development and general health. Pollen is being collected to assess potential fungicide biodegradation (right inset).