Fungicide Effects on Honey Bee Development

Project No.: **12-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] and may accumulate in hive materials [3-5] , it is unclear how closely those experiments resemble field exposures. In 2012, we peformed 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 is 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.

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, we are currently repeating these studies with a larger number of colonies. We are also focusing on one fungicide at a time, beginning with iprodione (Rovral). If this expanded study yields similar results to previous ones, we will consult with extension personnel to consider how best to reduce iprodione exposure to honey bees.

Materials and Methods:

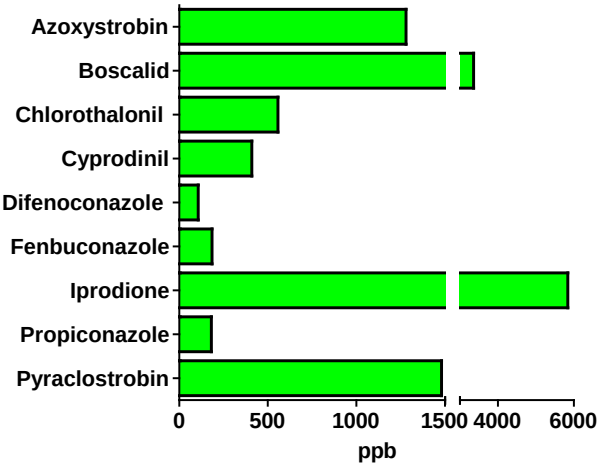

Figure 1. Fungicides in almond pollen. Maximum concentrations of fungicides found in pollen collected by honey bees in almonds in 2011 and 2012. 3 samples were measured in 2011, 5 in 2012

Pesticide concentrations: Iprodione concentrations were determined from pollen collected in multiple orchards in 2011 and 2012 in the Turlock area during almond bloom (**Figure 1**). Samples were analyzed by USDA/ARS in Gastonia, NC, 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.

Figure 2. Evaluating colonies frame by frame.Colonies we evaluated before treatment, and when flight cages were removed after treatment, as shown here.

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, OR) and transferred to standard 10-frame hives before the experiment.

Exposure: After initial evaluation (**Figure 2**), 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 Oregon State University Honey Bee Sagili laboratory.

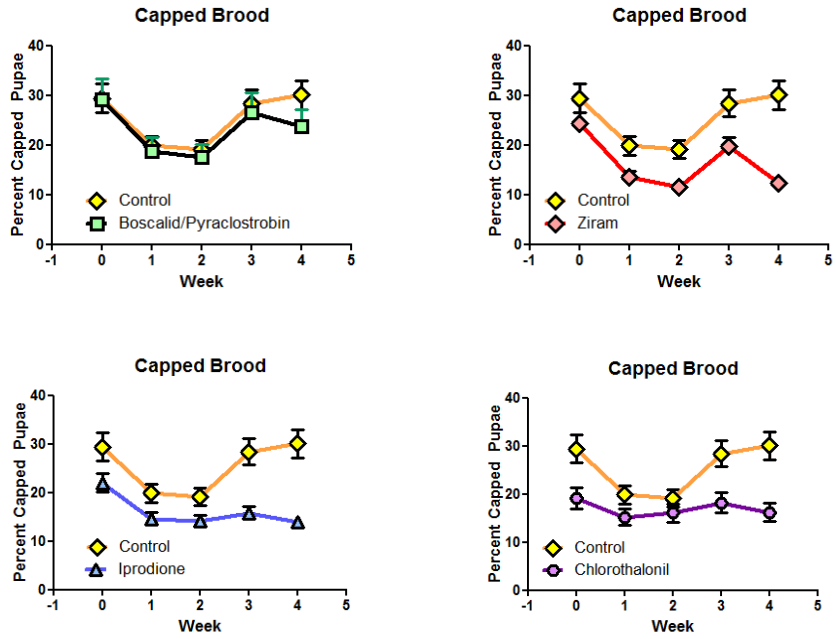

Figure 3. 2012 differences in production of capped brood between treatment groups. No difference was observed between control and boscalid/pyraclostrobin active ingredient treatments. Colonies treated with ziram showed a decrease in capped brood due to queen loss. The proportion of capped brood in iprodione and chlorothalonil treated colonies was significantly lower compared to controls.

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 [7], synergy between agrochemicals [8], 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 working our way through fungicides commonly applied during almond bloom to determine which if any contribute to this phenomenon.

In 2012 or 2013 we did not observe an increase in dead bees in front of hives, or any immediate colony-level effects in response to the fungicides we evaluated. In 2012, once evaluations were complete and we compiled our data to compared colony health over time, we made several important observations (**Figure 3**). It was not until the third and fourth week of evaluations that we observed a difference between control colonies and some treatment groups. We did not observe a significant difference between boscalid/pyraclostrobin (Pristine)

and controls. In the case of ziram, a decrease in eggs, larvae, and capped brood could be attributed to queen losses. Colonies treated with iprodione and chlorothalonil did not produce as much capped brood as control colonies. Differences between treatment groups were most clearly observed in capped brood, and variation in other measures reiterated the need for additional replicates.

In 2013 we have selected to further examine the effects of iprodione (Rovral) on honey bees. We have completed our evaluations, and are in the midst of compiling and analyzing this data. If our results are similar to 2012, we expect to see a decline in reproductive capability of treated colonies, most visible around week 3 and beyond. We are also assaying frozen samples from these colonies for other measures of colony health.

Possible molecular mechanisms underlying fungicide effects on honey bees are an open question. Although the timing of the observed effect in 2012 correlates with reports by beekeepers of effects on larvae 17 days after fungicide treatment, we did not observe larvae extended from their cells as described by others [1]. Fungicides are generally of low toxicity to adult bees [9], and if our results are confirmed, it is possible that fungicides affect fecundity of the queen, ability of adults to care for larvae, or development of worker bees from eggs to capped brood.

Fungicides may conceivably inhibit or imbalance the normal fungi and yeast communities in bee hives that ferment pollen into bee bread, which is reportedly necessary for bee nutrition [10]. 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 could also explain 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.

Together, our 2012 preliminary results indicate that certain fungicides may affect colony health, but may act indirectly, and effects may occur weeks after exposure. The complex relationships between possible pesticide exposure routes and multiple stages of honey bee development, and microorganisms responsible for fermenting pollen into beebread creates challenges in understanding potential impacts of fungicides. Using increased replicate colonies and multiple concentrations, our results from this season may help to confirm or validate the preliminary results for iprodione.

Research Effort Recent Publications:

None at this time.

Talks:

Fungicide Impact on Honey Bee Development
The Almond Conference
Effects of Fungicides on Honey Bee Development and Behavior
Oregon State Beekeepers Annual Meeting
Effects of Fungicides on Honey Bee Development and Behavior

American Bee Research Conference
Effects of Fungicides on Honey Bee Development and Behavior
Society of Toxicology

Posters:

Fungicide Impact on Honey bee Development
The Almond Conference

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