The Effect of Oxalic Acid Treatments on Queen Survival and Drone Semen Viability

Project No.: **08-POLL5-Ellis**

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Introduction:

In the spring of 2007, some beekeepers in the U.S. experienced severe unexplained colony losses. A team of scientists (Cox-Foster et al. 2007) identified the chemical burden of both in-hive and out-of-hive toxic chemicals as a potential contributing factor to colony stress, and they recommended further investigation of the role of toxins in the hive as a factor in predisposing colonies to collapse.

Honey bees are highly sensitive to most pesticides compared to other insects. This may be due to their habit of foraging for pollen and nectar, foods that contains few plant allelochemicals. Despite their sensitivity to pesticides, miticide treatments were the first line of defense when *Varroa* mites were found in the U.S. (Ellis 2001). Currently 8 miticides are registered for *Varroa* suppression in North America. While these miticides have been evaluated for worker bee toxicity, most of them have not been examined for toxicity to reproductive members of the colony. Likewise sub-lethal effects on drones and queens have not been investigated for most compounds (Desneux et al. 2007, Belzunces et al. 1993).

Since 1987, beekeepers have used a variety of miticides to suppress *Varroa* mite populations in their colonies. Some of the miticides used in beehives leave residues in hive products (principally beeswax) that could potentially interact with subsequent treatments synergistically to shorten the life span of worker bees, reduce sperm viability in drones, or to increase the frequency of supersedure in queens. The scientists conducting this study have previously investigated the toxicological properties of Oxalic Acid (OA) applied to worker honey bees and their *Varroa* mite parasite. This proposal would extend previous work on OA to determine if it affects queen survival and drone sperm viability. OA is low in costs, and it is a highly effective varroacide when applied to broodless colonies. OA is currently not registered and an investigation of reproductive effects will be an important contribution to OA registration, and it will provide scientists and beekeepers research-based knowledge about the bee safety of a promising varroacide.

Proposed Research:

We propose to examine the sub-lethal effects of OA on reproductive members of the colony, drones and queens. We propose to examine queen survival and productivity and drone sperm viability in OA treated queens and drones. The response variables we will measure for drones will be sperm number and viability. For queens we will examine queen longevity, egg laying rate, egg viability and brood production.

Procedures:

Sperm number and viability

- Establish LD_{50} for newly emerged drones and treat them with a sub-lethal dose of OA.
- Mark them and return them to their colony.
- Recapture the marked drones 14 days later and determine their sperm counts and sperm viability by dual florescent staining (Collins 2000).

Virgin queen survival in a queen bank

- Raise 180 virgin queens and conduct topical application bioassays for OA using the same 6 concentrations previously used for worker bees (Aliano and Ellis 2007).
- Place caged queens in queen banks and record daily mortality for 1 week.
- At the end of the trial, weigh all queens.

Mated queen survival, egg laying rate, egg viability and brood production in a colony

- Establish 45 mated queens in 3 frame nuclei.
- Divide colonies into 3 groups and treat with 2 concentrations of OA (Aliano et al, 2006).
- Record queen survival, egg production, egg viability and brood production.

Experimental design and data analysis

- Drone sperm viability and quantity will be examined in a completely randomized design using a t-test to compare outcomes. Data will be analyzed using SAS.
- Queen experiments will follow a completely randomized design. The least square means procedure in SAS will be used to analyze results.

Justification

- Some beekeepers are treating colonies with miticides as many as 3-4 times per year.
- Prior to 1985 the only agricultural chemicals used in beehives were antibiotics.
- Some miticides have been found to persist as residues in hive products (especially beeswax).
- Since beekeepers began putting miticides in their colonies, many have reported dwindling colonies, poor matings and high rates of queen supersedure.
- While the above effects may be due to biotic stress, such as Varroa mites, *Nosema ceranae* or bee viruses, they may also be due to chemical residues in hives or chemical interactions.

Results to date, mated queens study

To date, the only study completed is the investigation using mated queens. We placed mature queen cells in 45 three-frame nuclei. We divided the nuclei into 3 groups: untreated (1 μl of acetone), low dose (18 μl OA per μl), high dose (180 μl OA per μl). The high dose was equal to the LD_{10} for worker bees, and the low dose was 10 fold lower than the LD_{10}. When queens began laying eggs, they were narcotized with CO_2 and one of the 3 treatments was applied. Queens were then returned to their colonies and monitored for 8 weeks. Four response variables were measured for all colonies: queen survival, egg production, egg viability and sealed brood production.

Eggs Laid in 24 Hours

Treatment	Mean No. Eggs Laid(±SEM)	Mean Comparisons	*P* - Value
Control	315.7 ± 68.8	Control vs. High	0.350
High Dose	400.3 ± 58.7	Control vs. Low	0.420
Low Dose	387.6 ± 56.2	High vs. Low	0.870

Figure 1. There were no significant differences in the number of eggs laid during a 24 hour period. Means were compared using the least square means procedure.

Egg Viability

Treatment	Percent Egg Viability(±SEM)	Mean Comparisons	*P* - Value
Control	.835 ± .092	Control vs. High	0.004
High Dose	.467 ± .073	Control vs. Low	0.970
Low Dose	.841 ± .073	High vs. Low	0.001

Figure 2. There significant differences in the egg viability of high and low dose queens and of high dose and untreated queens. Means were compared using the least square means procedure.

Square Inches of Brood

Treatment	Mean No. Eggs Laid(±SEM)	Mean Comparisons	*P* - Value
Control	131.2 ± 75.9	Control vs. High	0.200
High Dose	92.3 ± 54.8	Control vs. Low	0.580
Low Dose	115.2 ± 67.25	High vs. Low	0.380

Figure 3. There were no significant differences in the square inches of brood 8 weeks after treatment. Means were compared using the least square means procedure.

Queen Survival

Treatment	Percent of Queens Surviving
Control	100%
High Dose	100%
Low Dose	100%

Figure 4. Queen survival 8 weeks post treatment with 2 doses of OA.

Conclusions and Discussion:

Once queens were mated and began laying eggs, no queens were lost or superseded during the experiment. The only variable that exhibited a significant response was egg viability with the high dose queens having reduced egg viability when compared with low dose and untreated colonies.

Queen exposure to a dose of OA that is higher than queens would normally be exposed to did not reduce egg laying or sealed brood production. However, it did result in a significant drop in egg viability. Since there were no differences in sealed brood production 8 weeks post treatment, it appears that the drop in egg viability may be a temporary condition that disappears over time. Treatment with a lower dose of OA that more closely resembles what a queen could be exposed to during colony treatment did not result in any reduction in egg laying, egg viability or sealed brood production. Neither the high dose nor the low dose reduced queen survival.

This study supports the recommendations that that beekeepers will need to be careful not to exceed the proposed recommended dose and that they should treat colonies when brood is not present in their colonies. In this study, excessive dosing and treating when brood was present reduced egg viability.

References:

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