Control of *Varroa* Mites in Honey Bees through the Systemic Application of Essential Oils

Project No.: 06-POLL8-Wardell

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Interpretive Summary:

The essential oils selected for this project effectively reduced mite populations in the honey bee colonies studied. The best method observed to introduce the oils into the colonies was through a protein/carbohydrate feeding supplement. Reformulation in the diet should improve absorption and transmission of the oils to the bee's hemolymph where it will impact the mites.

Objectives:

1. Screen oils in diets for palatability.
2. Compare volatiles emanating from larvae that have been fed diet containing essential oils with larvae not fed essential oils.
3. Determine the effects of diet + essential oil formulations on mite infestation and mite reproductive rates.

Materials and Methods:

Objective 1. Screen oils in diets for palatability.

The focus of this objective was to determine amounts of essential oils that could be put into diets without inhibiting feeding by bees.

The goal was to combine the essential oils with the liquid protein diet to create a product that would remain in suspension and would be palatable to bees. We tested a range of essential oil concentrations between 0.001% and 1.0%. We adjusted emulsification technique until a stable suspension of the oils had been obtained at the target percentages. Twenty grams of each essential oil concentration in diet was placed in open petri dishes on the top bars the colony. Bees had free access to the petri dishes. Plain bee diet (with no added oils) was used as a Control. Consumption was measured by weighing the dishes 1 hr. after placing it in the colony. Each petri dish series will be replicated five times.

Objective 2. Compare volatiles emanating from larvae that have been fed diet containing essential oils with larvae not fed essential oils.

The focus of this objective was to determine if there is a detectible change in the volatiles from larvae fed the test compounds as compared to the control treatments.

Colonies were provided with specific diets containing the essential oil and diet mix. Worker larvae from those colonies and control colonies were sampled when they were1, 3 and 7 days old. Five bee larvae from each treatment and control colony were placed in 5ml vials. A Solid Phase Microextraction (SPME) device was used to collect volatiles emanating from the larvae by placing the absorptive fiber into the vial containing the larvae. Volatiles from all specimens were collected onto the SPME device for 15 minutes at constant temperature.

Objective 3. Determine the effects of diet + essential oil formulations on mite infestation and reproduction rates.

The focus of this objective was to determine how well the selected compounds inhibit mite invasion and reproduction in the brood cells. We also examined whether the compounds affected mortality at specific honey bee developmental stages.

To determine the efficacy of the essential oil treatments, we monitored the rates of mite invasion and mite reproduction in the test colonies. Frames containing purple-eyed pupae were removed from each test colony at 30-day intervals. We recorded the: 1) number of cells with adult and immature *Varroa,* 2) the number of invading female (foundress) mites per cell, 3) the number of immatures per foundress mite, and 4) the number of dead mites in cells. We then determined the reproductive rate of mites invading cells by the equation: immature mites per cell / number of invading mites per cell.

Results and Discussion:

We successfully developed an emulsification process that would put the three different essential oils in a stable suspension. The best concentration for the oils was determined to be 0.01% active ingredient. Higher concentrations would cause repellency of the bees to the diet. At this concentration we got optimum consumption of

the diet and the greatest chance for the oils to be absorbed through the gut membrane and enter the hemolymph at concentrations that could potentially impact mite reproduction.

All test colonies consumed greater than 250 ml of diet oil mix each week. So we know the mix was getting into the bees of the colony. Through gas chromatography, we were to identify the oil in larval and prepupal samples. Significant levels of the oils were detected through SPME analysis.

Carrier Compatibility

The liquid protein honey bee diet developed by our team was reformulated to improve consumption. The new formula proved to be an adequate carrier for the emulsified oil suspensions.

Screen oils in diets for palatability

We selected three essential oils that are effective in limiting Varroa mite feeding and reproduction. By using a new emulsification process for getting the oils into suspension in the liquid protein diet, we were able to keep the oils in suspension indefinitely. We anticipate the reduced particle size in the emulsification will allow the oils to be easily transported across the honey bee gut membrane and increase oil levels in the hemolymph. Through the comparative feeding trials, we identified best concentration of oils in the diet to be 0.01% active oils ingredient. At this rate of application there is little repellency and no difference in consumption between the oil treated diets and the control diets.

Effects of diet plus essential oil formulations on mite infestation and reproduction rates.

Nucleus colonies were fed the diet with the emulsified essential oil over a three month period. Results of these trials can be seen in tables 1 and 2. After thirty days of being fed the diet and emulsified oils (indicated 1 and 2) the numbers of adult mites invading cells were significantly lower than in the controls. Table 2 depicts the reproductive rate of the mites over a three month period. A third oil was added to this experiment (Table 2) because of mite repellency activity seen in the laboratory, however, field results were not a definitive as seen in the laboratory. Over a three month period, mite reproduction in colonies treated with oils 1 and 2 showed steady and significant reductions in the ability of adult mites to reproduce. While mite reproduction in controls steadily increased, successful reproduction in colonies treated with oils 1 and 2 steadily decreased until the reproduction rate fell below 1.0, a point at which mite population are in steady decline because they can not replace the adults.

Comparison of larval volatiles

Comparative analysis of volatiles emitting from larvae fed control and treatment diets demonstrated that the oils were entering the larval food chain. While the amount of volatiles emanating from the larvae was low it was definitely present. Examining the volatile data along with the mite reproduction data it is evident that the oils in the diet mix significantly reduced mite numbers in the cells and mite reproduction in the colony (Table 2). The study was conducted over a three month period. While results were very promising, it still took nearly three months of treatment to accomplish the greatest

results. This is too long a time to affect control for practical commercial applicability. We believe through either greater consumption, increased concentration without repellency or more effective emulsification of the oil we could affect mite control in a timelier manner.

Table 1. Mite Reproduction in Colonies Fed Essential Oils

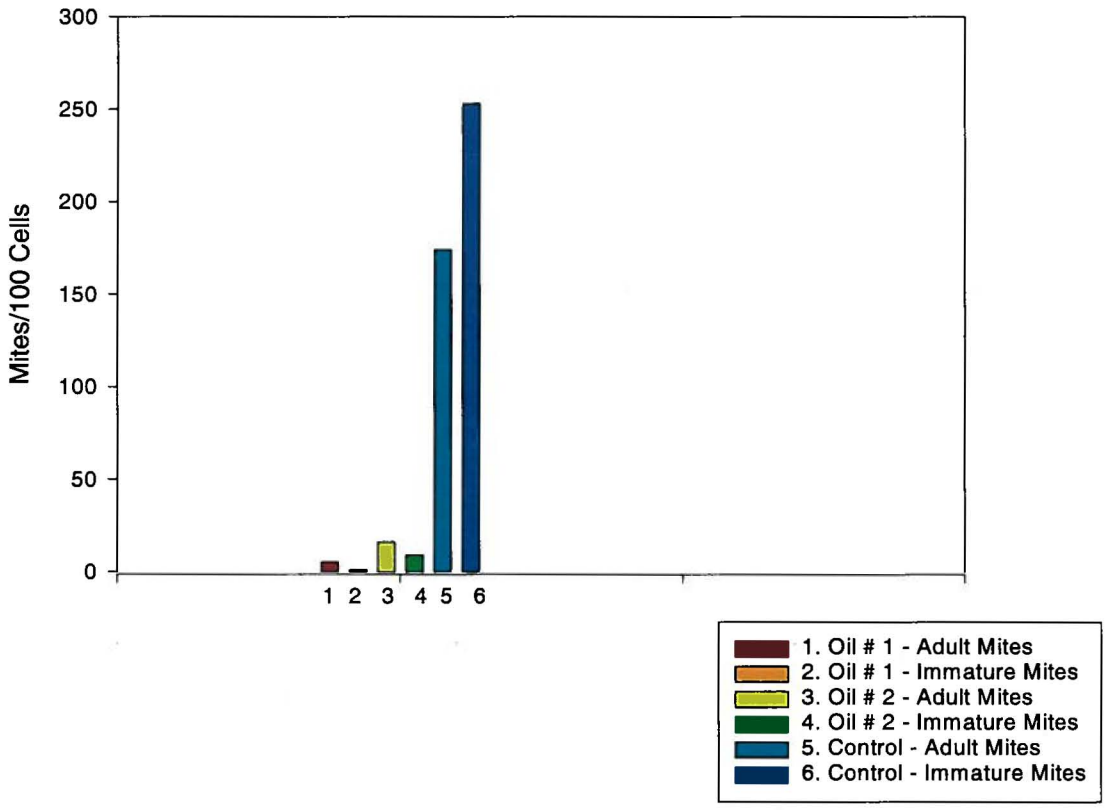


Table 2

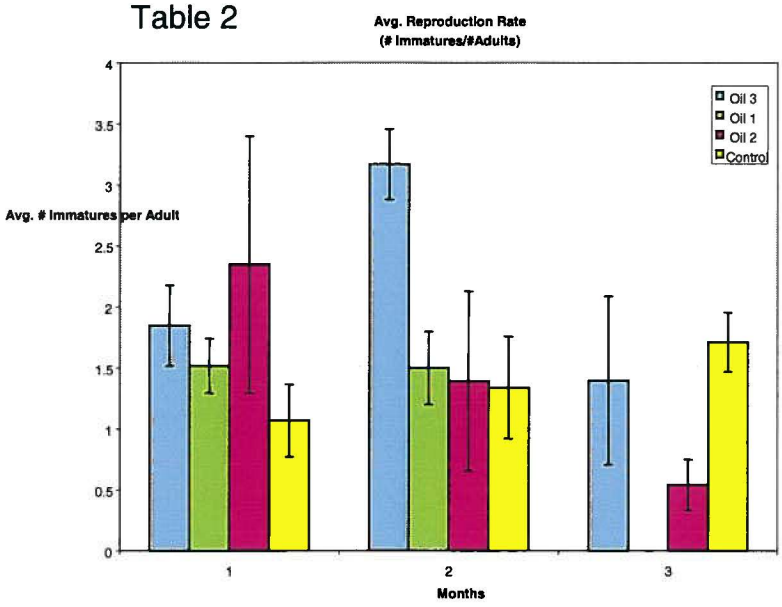
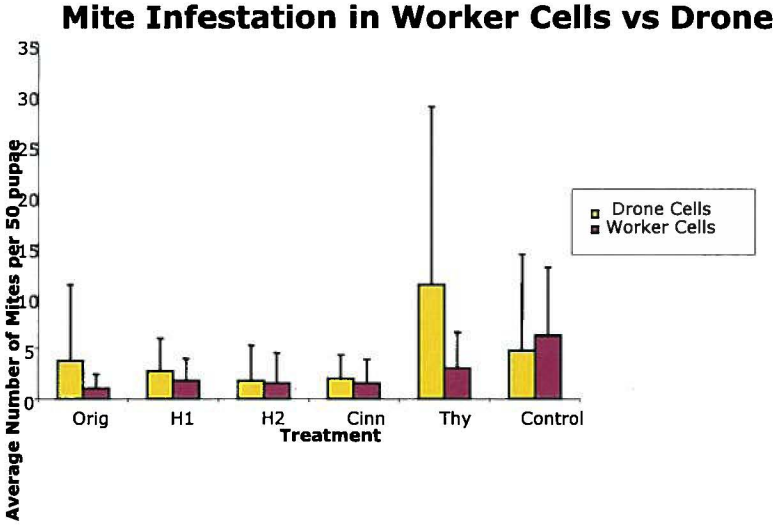


Figure 3



H2 and cinnamon did the best in controlling mite infestation in both worker and drone cells. The data demonstrate that mite levels can be significantly reduces with the systemic application of essential oils. With the improved delivery system it may be possible to stimulate brood production in colonies at the same time as controlling mites.