Year End Report
Project Year 2004

Project No: 04-GW-01

Project Title: Control of *Varroa* mites through the systemic application of compounds mixed in a liquid honey bee diet.

Project Leader: Dr. Gordon Wardell, and Dr. Diana Sammataro, USDA, ARS Carl Hayden Bee Research Center, Tucson AZ

Location: **Carl Hayden Bee Research Center, USDA ARS, 2000 E. Allen Rd, Tucson AZ**

PROJECT GOALS

The goal of this project is to limit *Varroa* mite reproduction by developing a means to deliver specific essential oils and volatile compounds to honey bee larvae through the nurse bees. Unlike the volatile delivery systems for essential oils we will be feeding the oils systemically to the bees and in effect mimicking the resistance seen in the SMR and Russian bees. To accomplish this, we will be combining two technologies developed between S.A.F.E. R&D, LLC and the Carl Hayden Bee Research Center to produce novel *Varroa* mite control products. Essential and naturally occurring oils as well as bee host volatiles will be selected for their ability to control parasitic mites by limiting the mite's ability to feed on the larvae when the compounds are fed to larvae via the nurse bees.

SUMMARY

In an effort to systemically control *Varroa* mites in honey bees, we first analyzed the volatile compounds found in mite susceptible and mite resistant lines of bees. The difference in chemical signatures we found will hopefully provide clues to assist in developing a systemic treatment for *Varroa* using naturally occurring essential oils. When screening essential oils for bioactivity against parasitic mites, we were able to identify four highly effective essential oils or derivatives of essential oils to use in this project. We were successful in developing an emulsification procedure to get the essential oils into a carrier that was fed to the larvae and ultimately impacted mite reproduction. The first carrier we tried was a sugar syrup solution. While consumption of the syrup/oil mixture was high, almost no perceivable amounts of the oils could be found in the larvae and consequently the treatment had almost no impact on mite reproduction. As a follow up experiment, we emulsified the essential oils in a newly developed liquid protein diet for honey bees. The new diet proved to be a compatible carrier and, in a preliminary experiment, mite reproduction levels were significantly reduced. Experiments will be refined and expanded in the next field season to determine the mode of action of these oils in the liquid protein diet.

OBJECTIVES

The specific objectives of the project are:

1. Volatile compound identification from larvae of resistant lines of honey bees.
2. Screening of essential oils to be used in Varroa reduction trials.
3. Determine the maximum level of compounds (oils and compounds from resistant lines of bees) that can be incorporated into diet and not reduce palatability to honey bees.
4. Quantify these compounds in the gut and hemolymph of treated larvae.
5. Determine the rate of mite invasion and reproduction in pupal cells of bees fed the treated diet and controls.

Objective 1. Volatile compound identification from larvae of resistant lines of honey bees.

Volatiles from five larvae of resistant and susceptible stock (age 3 to 7 days) was sampled. Information about the lines of bees can be found in the final report *Investigating Volatiles for Possible Varroa Mite Control.*

Results

We have begun to compare retention times of (indicating peaks of interest). To eliminate the background fiber peaks and other noise, we selected particular ion fragmentation ion numbers that correspond to hydrocarbons and similar chemical families. In this way, we can clarify the chromatogram plots, eliminating unwanted peaks, such as fiber or machine material, making the plots easier to read. Each day, we run a blank air sample to compare with our real samples.

We sampled a Day 12 Russian drone larva and its mites. A D12 larva is just beginning to spin the cocoon and it is at this stage that the larva is most attractive to foundress Varroa mites. All compounds recorded thus far have been of hydrocarbons. We sampled an infested Russian drone pupa (Day 18) separated from the mites, and compared that with the immature mites from the same cell, mature female mites from that pupa and mite frass found inside the cell. As expected, many of the peaks are similar (mite and bee), as mites feed on drone hemolymph (blood) and defecate in the cell. There are some unique drone peaks that show up when the drone is run separate from its mites which we are still analyzing.

A susceptible bee line was also sampled. This strain is raised in Hawaii and has not had the selection pressure to be resistant to mite infestation. As a result, the bees are highly susceptible to mites, especially the drones. We selected an infested D12 drone prepupa larva and compared it with a D12 uninfested larva. We did find one unique peak in the infested bee. We then chose D20 pupae, about four days before they emerge. At this age, we will get the most number of mites per cell, which includes the foundress mothers and their offspring. The uninfested drone had much higher compound activity (higher peaks) than the infested drone and its mites. Out next step is to make a more detailed assessment of interesting peaks that can be used for future research on the effects of these compounds on mite reproduction

Objective 2. Selection of essential oils to be used in *Varroa* reduction trials.

We selected four essential oils based on earlier laboratory and field trials. The oils were known to have an impact on *Varroa* mites and were palatable to the bees when prepared at the proper concentrations. The oils demonstrated bioactivity in controlling *Varroa* mites and had no secondary effects in the colonies. The oils did not disrupt normal colony behavior nor did they exhibit any sub lethal impact on the adult or immature bees in the colonies. The oils selected were: Origanum, Thymol, Cinnamon oil, and 2 Heptanone.

Objective 3. Determine the maximum level of compounds (oils and compounds from resistant lines of bees) that can be incorporated into diet and not reduce palatability to honey bees.

The maximum level of oils fed to the colonies was determined by performing feeding preference trials at various concentrations and comparing those results with controls. We determined the best level of oils to incorporate into the test diet was 0.01% active ingredient. At this level we found adequate availability to the bees and yet did not cause any feeding inhibition. Higher level of the oils caused repellency and resulted in reduced consumption.

Objective 4. Quantification of the target compounds in the gut and hemolymph of treated larvae.

After the treatment colonies have been fed the experimental diet for one week, six day old larvae will be pulled from their cells and five microliters of hemolymph will be collected to analyze. The hemolymph will be placed in a 2 ml sterile vial where it will be exposed to a Solid Phase Micro Extraction fiber (SPME) which will then be sampled in a Varian GC Mass Spec.

Results:

The visualizations of volatiles from the four essential oils fed in sugar syrup using SPME was not strong. We compared each compound in newly hatched bee larvae (Day 4 from being laid) and Day 9 larvae, which are larvae about to be capped over with wax, and most susceptible to Varroa attack.

For compound A (Origanum) the material was detected but only in very low counts (see Fig. 1). We ran several other comparisons (see appendix). We determined that the compounds were either too low to detect from being fed in sugar syrup or that we needed to refine our SPME protocol.

As a result of our investigations, we not only changed the feeding protocol to incorporating the essential oils in the diet, but we also refined our SPME analysis to make it more sensitive to detecting these volatiles in the diet. This is what we are now working on, and will be included in future work.

Objective 5. Determine the rate of mite invasion and reproduction in pupal cells of bees fed the treated diet and controls.

The focus of this objective is to determine if the sugar syrup solutions infused with essential oils have any impact on either the mite invasion of cells or the reproduction of the mites once they enter the cells. The principle is to have the oils/diet mix fed to the larvae by the adult bees and once in the larval gut the oils will be absorbed through the gut membrane and eventually be incorporated into the larval hemolymph. If the oils deter the mites from feeding, the mites will not reproduce. The first series of feeding trials were conducted with a sugar syrup carrier. The emulsified oils were mixed into sugar syrup and fed to the bees through a division board feeder. A subsequent experiment was conducted with a newly formulated liquid protein diet as a carrier.

Results:

While the emulsified oils mixed well, stayed in suspension, and were readily consumed by the bees, little of the compound was found in the larval samples. Mite infestation rates were no different in the oil and sugar syrup treatments than they were in the controls. Mite reproduction was also not impacted by the introduction of the oils.

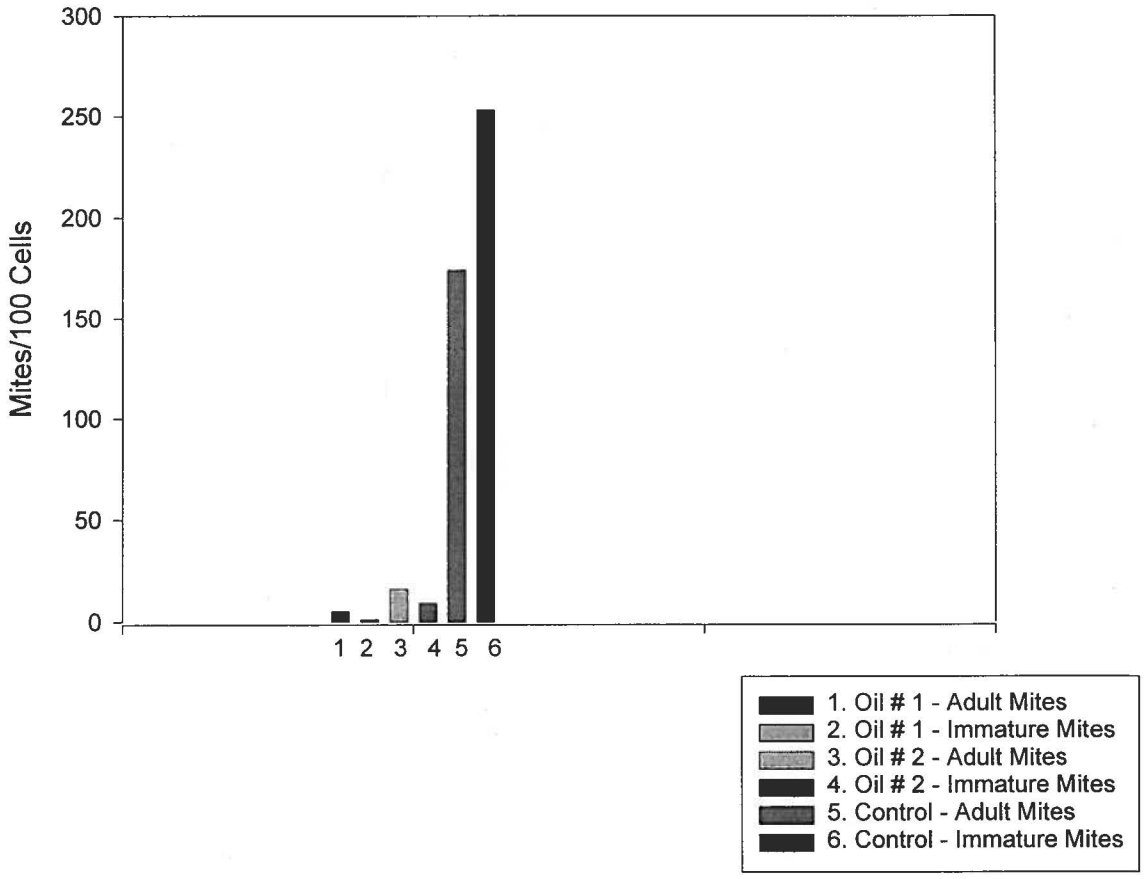
Following the feeding trials with sugar syrup we decided to try the newly developed liquid protein diet as a carrier for the essential oils. This diet proved to be an excellent medium for the essential oils. A preliminary experiment demonstrated that when the oils were mixed in the diet mite reproduction fell precipitously (see Table 1). Mite reproduction was well below the level that would support mite growth in a colony. The experiment was very successful and follow up feeding trials will be conducted in the near future.

DISCUSSION:

We determined through the experiments conducted under this grant that when essential oils are emulsified in sugar syrup solution and fed to the bees, the oils are not typically fed to the larvae. Results indicate the syrup/oils mix is stored in the combs but does not get mixed into the larval food. The challenge then became to find a carrier for the oils that the bees will eat and feed to the larvae. In a preliminary experiment where emulsified essential oils were suspended in the newly developed liquid protein diet (MegaBee ™, S.A.F.E. R&D) mite reproduction was significantly reduced when compared to controls (Table 1). The next step will be to determine the mode of action of the oils in

the diet. We plan to use gas chromatography as described in objective 4 to determine whether the oils are present in the larval gut, hemolymph or on the cuticle. Determining the mode of action will enable us to improve refine the delivery system.

Table 1. Mite Reproduction in Colonies Fed Essential Oils



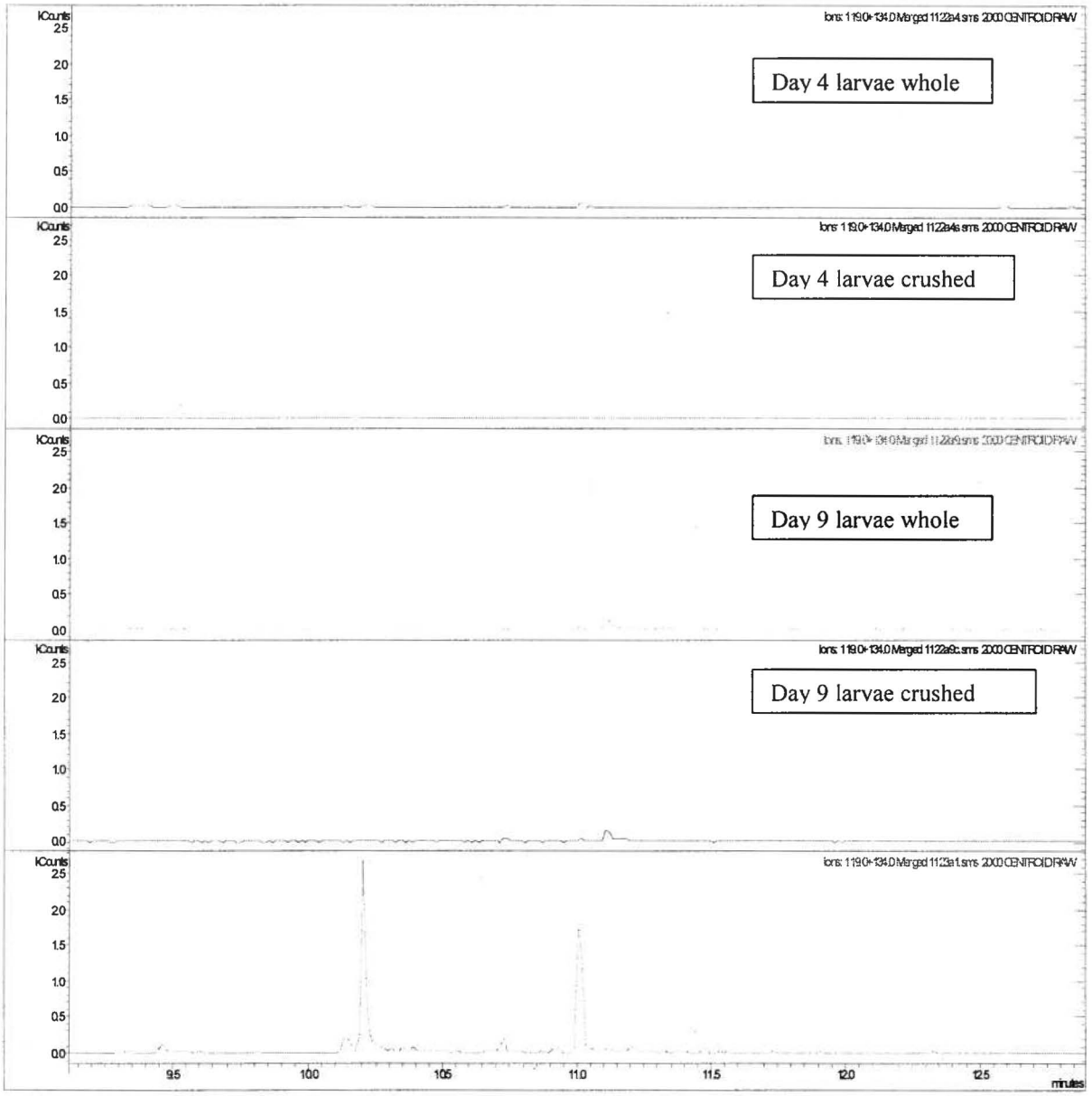


Figure 1. Compound A (Origanum) Chromotogram. The bottom chromatogram is the air blank which too high peaks for the amount of origanum in the samples. As can be seen in the chromatograms little or no essential oils were found in the treated larvae.