Essential Oils: New Methodology to Control *Varroa* Mites

Project No.: 06-POLL1-DeGrandi/Ahumada

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Interpretive Summary:

The ectoparasitc mite *Varroa jacobsoni* is the most destructive pest of honey bees *Apis mellifera*(10,11).The rapid emergence and spread of the parasite in the last 10 years has drastically changed beekeeping practices. To avoid the devastating effects the mites have on honey bee colonies, beekeepers are forced to treat mite infestation with pesticides. The repeated application of such products can lead to a potential problem of residues in apiculture products, to a lesser extent in honey and also the elevated chance of environmental contamination (6). The rapid development time of *Varroa,* the large population and the massive use of chemicals are contributing to the development of resistance, especially if challenged repeatedly with the same pesticide(4). With the increasing resistance of mites to registered acaricides, there is an immediate need for alternative methods to control honey bee parasites. Last year, under Almond Board grant project number **05-GD-01**, we successfully screened, identified and developed a new and novel essential oil delivery system based on a starch microencapsulation. In this process, cornstarch was used to encapsulate microscopic droplets of the oils about the

size of a pollen grain. The present proposal is a continuation of the investigation described above. The long-term goal of this research project is to develop an efficient and accurate delivery system for the utilization of plant essential oils to control *Varroa* mites. Our goal is to find three essential oils that are effective in controlling *Varroa*, so in the end, we will be able to provide products that can be alternated to minimize the chance of resistance.

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Objectives:

The main focus of this research project was the delivery system of the microencapsulated essential oils, which we have shown to be very effective in controlling mite population.

The project objectives were as follows:

1. Reformulate the delivery system to improve the release rate of the encapsulated oils.
2. Field trial to assess the new formulations.
   a) Feeding preference
   b) Mite monitoring
3. Gas Chromatograph analysis on treated colonies.

Materials and Methods:

The experimental design for each objective is described below:

Objective 1. Reformulate the delivery system to improve the release rate of the encapsulated oils. As we described in previous reports, the microencapsulated essential oils have proven to be very effective in controlling *Varroa*. The microencapsulation of the essential oils was done by ARS chemists at the USDA Bioproducts Laboratory in Albany, CA. The chemists have developed a technique for microencapsulation that we used for our essential oils as a delivery system. The active ingredient (AI) of the oil in the capsules was 25%. In order to achieve a better effect when applying the oils to the colony, the ARS chemists worked on a new delivery system. Basically, it consisted on a thin sugar-starch strip saturated with essential oil. To attain this effect, the strip was formulated using different microtubules in a range of sizes and diameters that allowed the oil to be released at various rates. The object with this delivery system was to have a quick release of the oil at the beginning of the treatment in order to create a highly concentrated atmosphere in the colony to kill as many mites as possible and a slow steady release afterwards.

The essential oils proposed to be tested were: Clove, Cinnamon, Thymol and Origanum.

Objective 2. Field trial to assess the new formulations. The focus of this objective was to determine the optimum concentration of the oil to be used and the efficacy to control *Varroa*.

a) Feeding Preference

The sugar-starch strips saturated with oils were positioned on the top bars of the colony. Each strip had approximately 20% of active ingredient. Plain sugar-starch strips without oils were placed in colonies that were used as control. Due to the high concentration of oil in the strips at the beginning of the treatment that caused repellency we were not able to measure consumption. The strip weight was recorded before and after being placed in the colony and data was collected weekly.

b) Mite monitoring

The main focus on this objective was to determine and compare how well the selected starch encapsulated compounds in the starch-sugar strip can inhibit mite invasion and reproduction in the brood cells.

Pre-treatment mite population levels in 25 5-frame nucleus colonies were determined by the sticky board technique. The duration of the treatment with the microencapsulated oils was 6 weeks followed by 1 week of Apistan, a commercially available miticide used as a known treatment to kill the remaining mites in the colonies. The results and data analysis from these experiments are shown in Figures 1 through 4. Each delivery system was fed to 5 nucs for each treatment period. Control nucs were fed plain sugar-starch strips without the essential oil.

The following Figures 1 to 4 show the total number of mite drop weekly in colonies treated with starch-encapsulated strips containing different essential oils. The oil strips had a very high concentration at the beginning causing a repellency effect that was overcome for Origanum and Thymol after a few days. These treatments showed efficacy in mite drop and Thymol showed to be the most effective in controlling mite population in the colonies. In the case of Cinnamon, the concentration remained high throughout the treatment period and could not overcome repellency. The treatments were carried out for 6 weeks followed by an Apistan treatment at the end to remove all the remaining mites in the colonies.

Figure 1: Weekly Mite Drop with Thymol

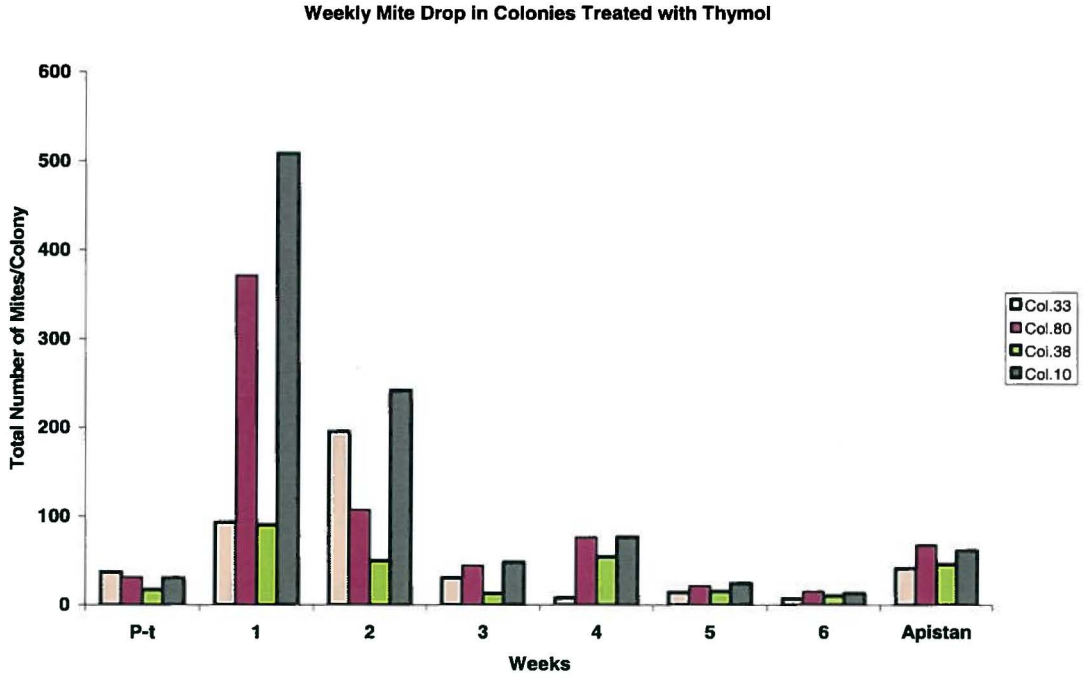


Figure 2: Weekly Mite Drop with Origanum

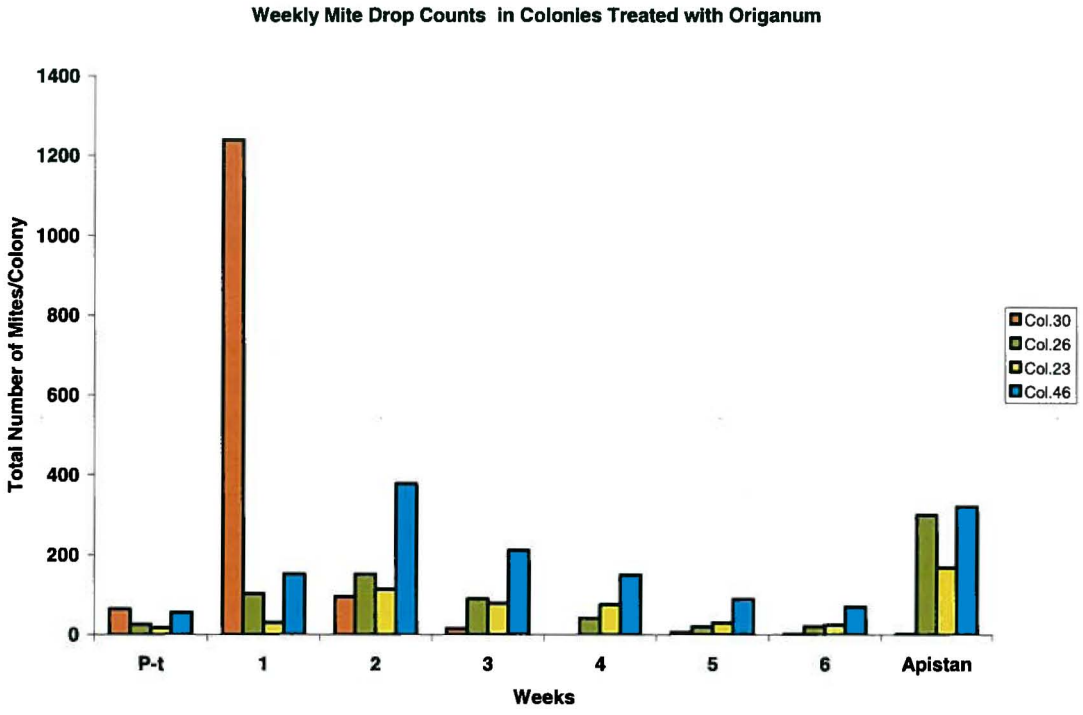


Figure 3: Weekly Mite Drop with Cinnamon

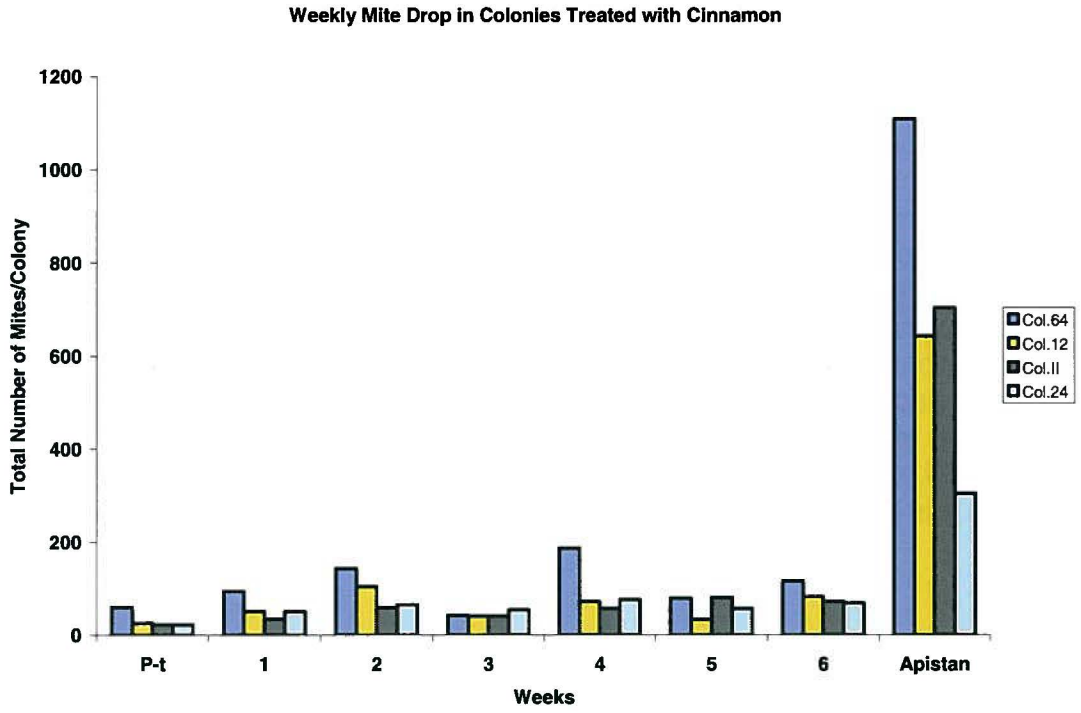
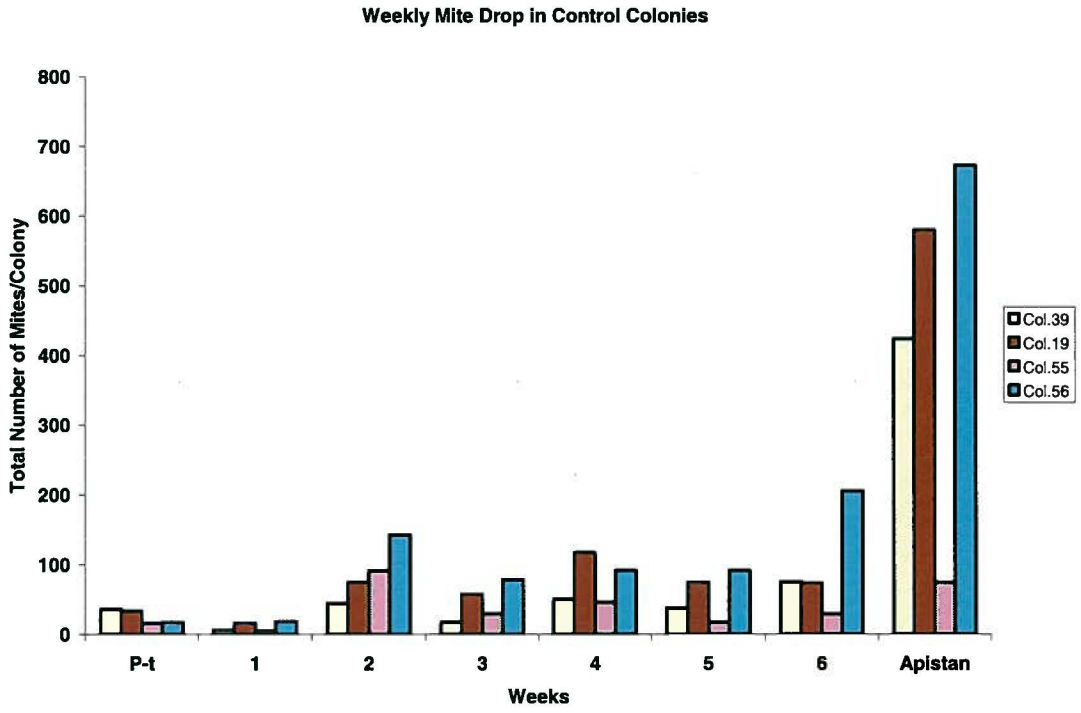


Figure 4: Weekly Mite Drop in Control

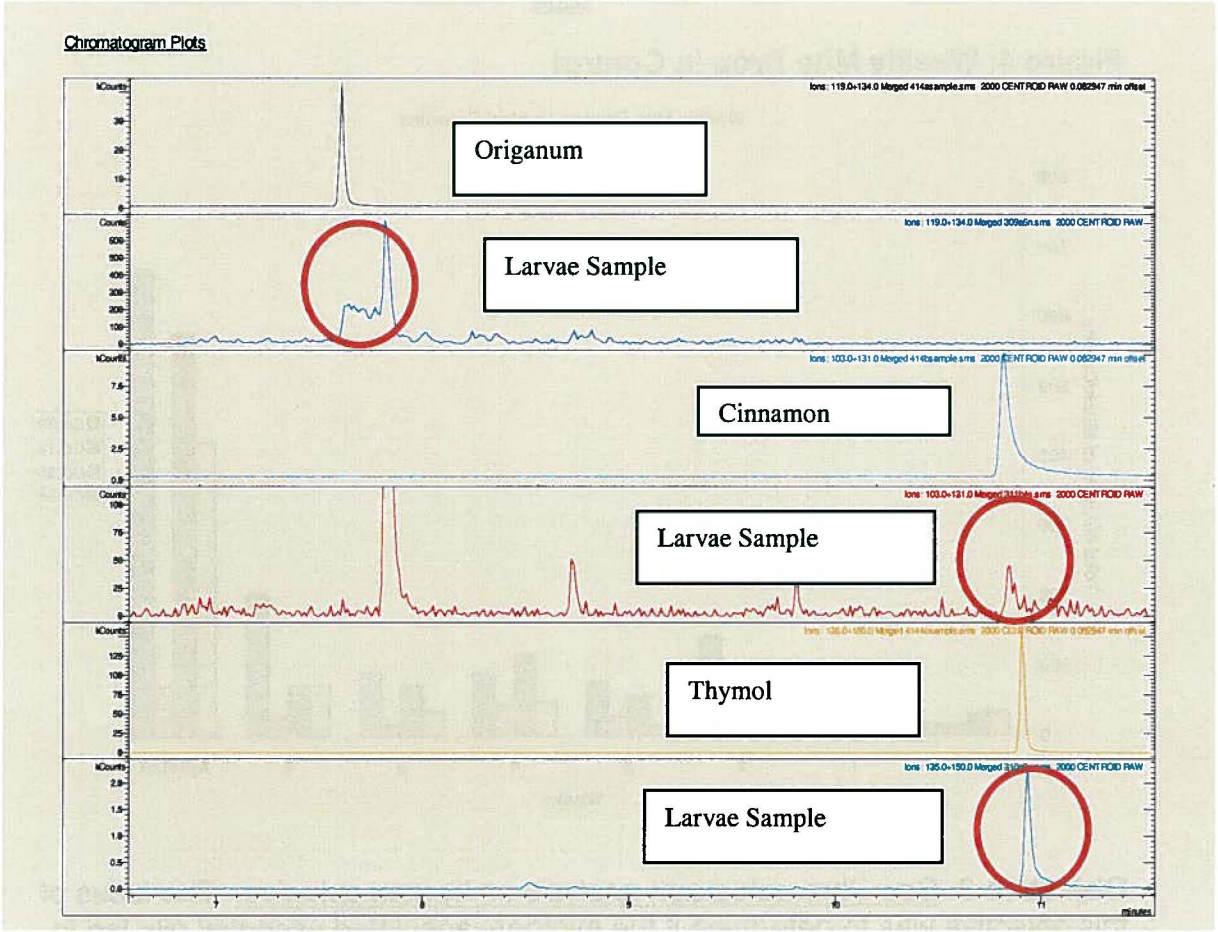


Objective 3. Gas Chromatograph analysis on treated colonies. The focus of this objective was to determine if the microencapsulated essential oils fed to the larvae can be detectable and be compared to the control treatments. Worker larvae at 1, 3 and 7 days old that were fed microencapsulated essential oils in the strip were sampled. Five honey bee larvae from each treatment and control colony were placed in 5 ml glass 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 sampled for 15 minutes at a constant temperature.

Volatile samples from the larvae weree analyzed using a Varian Model 2200 Gas Ghromatograph/Mass Spectometer (GC/MS) to separate and identify volatile compounds. The SPME fiber will be desorbed at 240°C for 3 minutes and the components separated on a Varian VR5ms column. The compounds were tentatively identified using the on-line MS library (both Wily and NIST are available). The chemical profile of treated and control larvae at the same developmental stage were compared and analyzed using the chemical library described above. Concentrations of compounds will be determined through relative abundance analysis. Dr. Judith Hooper of Pima Research and USDA CRADA partner will be conducting the GC analysis. Results and data analysis from this experiment are shown in Figure 5.

Figure 5: Chromatogram Plots



Chromatogram Plots performed on larvae were able to show traces of essential oils were present on the larvae from treated colonies, meaning that the oils were fed to the larvae and have worked in a systemic phase.

Results and Discussion:

- The delivery system using starch encapsulated oil strips seemed to be an easy and effective method to be applied for mite treatment. Even though the strips need to be modified to assure a more steady release rate, it is a very promising delivery system that can be used by beekeepers.
- Due to the high concentration of the oils at the beginning of the treatment, some repellency effect was shown in Cinnamon, Thymol and Origanum. The effect was overcome in the later oils after a few days.
- From all the oils tested, Thymol and Origanum showed to be effective in controlling mites in the treated colonies. The strips were moved around the colony and the bees were able to get in contact with them.
- Overall the starch strips containing Thymol shown to be the most effective.
- The strips were able to show efficacy up to 6 weeks in decreasing mite population.
- Cinnamon strips showed a very high repellency level over the 6 weeks treatment not allowing the bees to get direct contact with the strip.
- As described in the Chromatogram plots, traces of the oils were found in larvae meaning that they have been fed or in contact with oils.
- Even though the delivery system works well, we need to improve the release rate of the oils and we plan to do that as an extension of this research project.

The potential benefit and outcome of this proposal along with the experimental design, results and data statistical analysis was presented at the Almond Board Conference 2006 in both oral and poster presentation.