Essential Oils: New Methodologies to Control *Varroa* Mite

Project No.: **07-POLL2-DeGrandi/Ahumada-Segura**

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Interpretive Summary:

During the past couple of years, we have been working on a variety of delivery systems for essential oils. Working in conjunction with ARS chemists, we were able to show that microencapsulated essential oils have proven to be very effective in controlling *Varroa* mites. We have tested the encapsulated oils in a free flowing powder as well as in a solid strip. Results showed that oils contained in micro-capsules provided a better way of releasing the treatment in the colony. Our previous research with microencapsulated oils demonstrated that Thymol and Clove were very effective on decreasing the number mites in the colony. Treatments were applied weekly and mite levels were monitored during a 6 week period.

The main focus of the research project during 2007-2008 was on the delivery system of the microencapsulated essential oils, mainly in the contact and vapor phase using the oils that have shown to be very effective in controlling mite population. To determine the efficacy of the treatments, we monitored the rates of mite invasion and reproduction in the test colonies as well as bee toxicity and behavior. During the final phase of the research the essential oils were applied in the colonies using two delivery systems.

One method was applying free flowing powder mixed in with powdered sugar at 2.5 % active ingredient and the other method by using an edible sugar pouch filled with free flowing oil powder at 16% active ingredient. The essential oils utilized for the treatments were: Clove, Thymol Camphor and Neem oil.

The data collected from the trials showed that both delivery systems are effective in controlling the mite population in the colony. Although they need some improvements they have proven to be an alternative for mite treatment.

Thanks to the funding provided by Almond Board to conduct this research we have been awarded two SBIR (Small Business and Innovation Research) grants, Phase I and Phase II to continue the research on microencapsulated oils for mite control. We are going to focus on improving the delivery and dispersion of the encapsulated oils in the colony.

Objectives:

The project objectives were as follows:

1. Reformulate the Delivery System of the Starch Strips to Improve the Release Rate of the Encapsulated Oils:
   a) Contact phase delivery method
   b) Vapor phase delivery method
2. Field Trial to Assess the New Strip Formulations:
   a) Bee Toxicity
   b) Mite monitoring
   c) Bee behavior

Materials and Methods:

Objective 1: *Reformulate the Delivery System of the Starch Strips to Improve the Release Rate of the Encapsulated* Oils

As we described in the interpretive summery, the microencapsulated essential oils have proven to be very effective in controlling *Varroa*. Preliminary results using starch encapsulated strips showed very low efficacy of the delivery system and the strip application was replaced by an edible pouch. In this objective two delivery systems were tested: a reformulated free flowing powder mixed in with powdered sugar and an edible sugar pouch filled with free flowing oil powder. A combination of different materials and particle sizes was utilized in this process.

a) Contact Phase Delivery Method:

Free flowing microencapsulated oil powders were placed inside an edible sugar pouch. The pouch was sealed and kept at room temperature until introduced in the colony. The object of this delivery method was to have a controlled release mechanism of the oils that it will be regulated by the bees itself by opening the

pouch and getting direct contact with the powder. The powder will be transferred from bee to bee inside the colony.

b) Vapor phase delivery method:

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 free flowing powder was mixed in with powdered sugar for the applications and proper adjustments were made in order to accomplish the proposed objective. A combination of different materials and particle sizes to formulate the powder was taken into consideration as well.

Proposed Essential Oils to be Tested

Based on their miticide activity, we have selected: Clove, Thymol, Camphor and Neem Oil.

Objective 2: Field Trial to Assess the New Formulations

The focus of this objective was to determine the efficacy of the oils to control *Varroa* and also monitor bee toxicity and behavior.

1. Bee Toxicity: The starch-encapsulated oils as a free flowing and in the pouches were positioned on the top bars of the treatment colonies. In order to determine if the new formulations of the oils were toxic to the bees, dead bee traps were placed in each colony. Daily dead bee counts were collected and mean mortality recorded.

2. Mite Monitoring***:*** The main focus on this objective was to determine and compare how well the selected starch encapsulated oils in the pouches and as free flowing powder can inhibit mite invasion and reproduction in the colony. Pre-treatment mite population levels in 25 5-frame nucleus colonies (nucs) were determined by the sticky board technique. Each delivery system was placed in 5 nucs for each treatment period. Control nucs were treated with plain starch strips without the essential oils.

3. Mite Infestation and Reproduction - To determine the efficacy of the essential oils treatments, we monitored the rates of mite invasion and reproduction in the tests nucs in both delivery systems. Frames containing purple-eye pupae were removed from each treatment nuc at 21-day intervals, which correspond to the development time of the workers from egg to adult. Fifty brood cells will be uncapped and the pupae removed.

In this experiment we recorded:
1) The number of cells with adult and immature *Varroa* mites.
2) The number of invading female (foundress) mites per cell.
3) The number of immatures per foundress mite in the cells.

4) The number of dead mites in the cells.

We analyzed the data to determine the reproductive rate of mites invading cells. The equation used to determine mite reproduction will be: immature mites per cell/number of invading mites per cell.

We conducted a one-way analysis of variance to determine whether significant differences in the percentage of cells infested and the average number of mites reproduced per female exist within the same nucs before and after treatment and between treatment and control nucs.

4. Bee Behavior - Treatment and control colonies were monitored very closely to determine if the presence of the starch-encapsulated oils have an impact on disrupting bee behavior and colony performance. The observation of any significant changes in the colony was recorded and proper adjustments if necessary were made.

Results and Discussion:

The treatments with free flowing powder and edible sugar pouch were applied to the colonies and carried out for 4 weeks followed by an Apistan application at the end to remove all the remaining mites in the colonies. The powder treatment was applied weekly to ensure a continuous exposure to the oils via systemic and contact. The pouch treatment was applied at once and bees consumed the edges of the pouch until the powder inside was exposed. The transfer and dispersion of the powder inside the colony had an impact on the mite population. Mite drop in the pouch treatment was low at the beginning and increased considerably as the powder coming out of the pouches augmented. Mite drop in colonies on this delivery system reached a spike at week 4 of the treatment. Although edible pouch Neem treatment was very effective in dropping mites the colonies under this treatement showed abnormal bee behavior and high bee mortality was observed at the end of the experiment. The concentration of the oils contained in the pouch was probably too high and needs to be adjusted for future trials.

The following Figures 1 to 4 show the total number of mite drop weekly in colonies treated with both delivery systems. The mode of action of both treatments can be described as vapor, contact and systemic.

Figure 1.

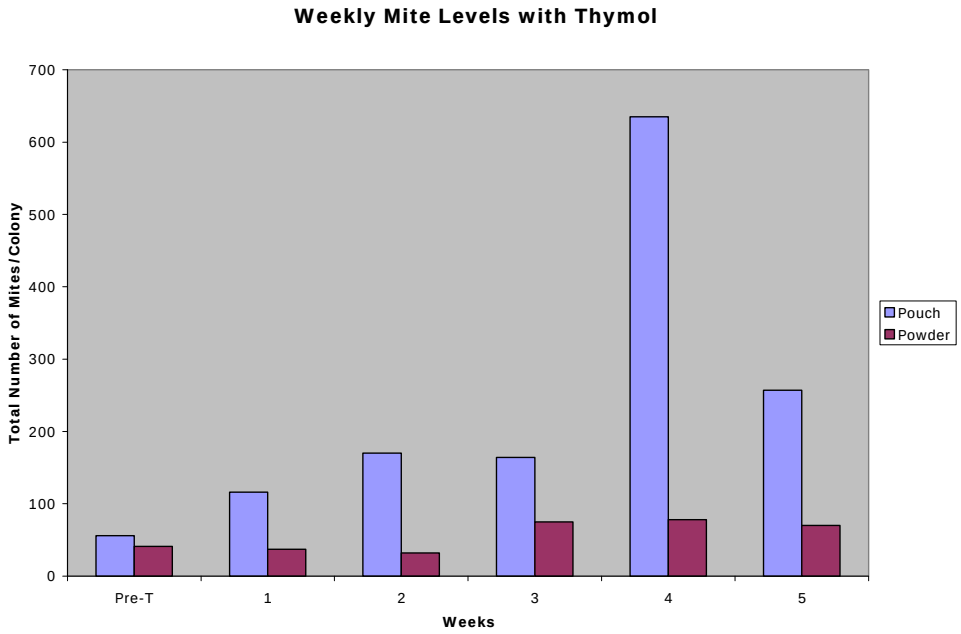


Figure 2.

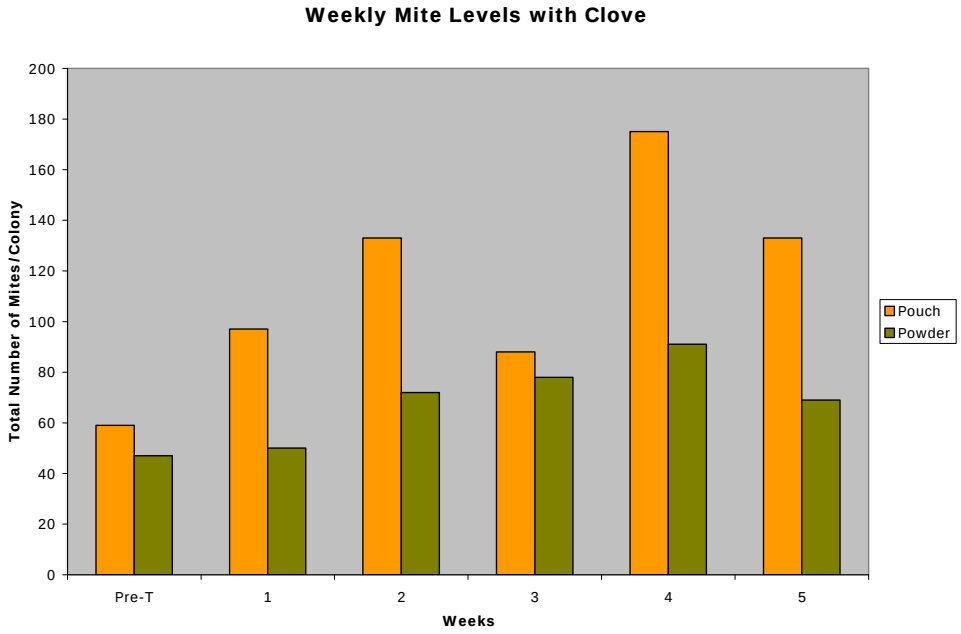


Figure 3.

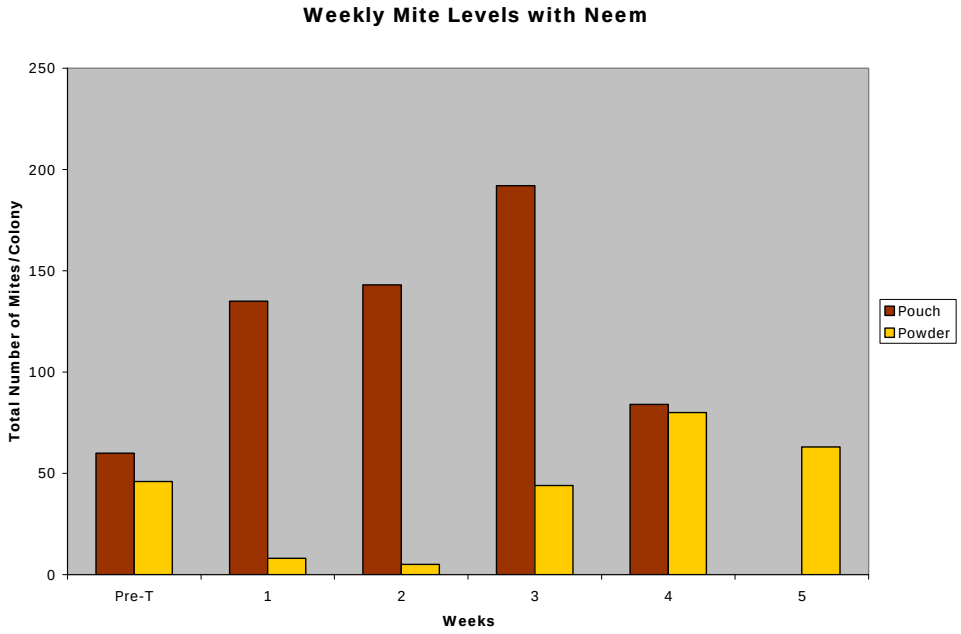
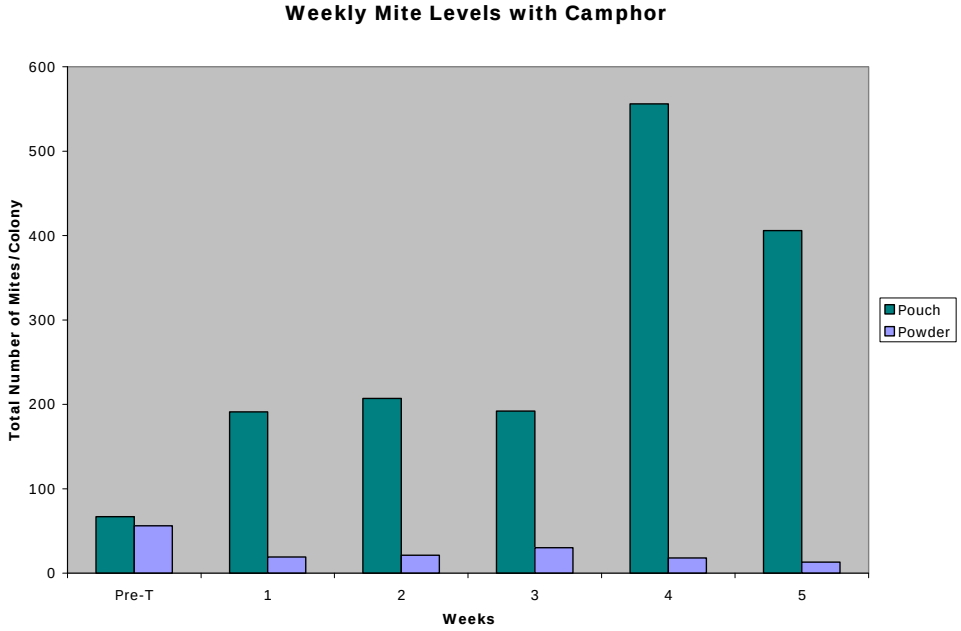

Weekly Mite Levels with Neem
Total Number of Mites/Colony
250
200
150
100
50
0
Pre-T
1
2
3
4
5
Weeks
Pouch
Powder

Figure 4.



The delivery systems and the essential oils tested under this research showed tremendous potential as alternative methods for *Varroa* control. The microencapsulated formulations need to be adjusted to avoid bee toxicity and bee behaviour changes. The Almond Board funding enabled us to perform the research using these innovative delivery systems and test a wide range of treatments through vapor, contact and systemic applications. The data collected allowed us to continue the research by improving the delivery system through funding awarded from SBIR grants.

The use and application of starch encapsulated essential oils offer great advantages such as: bioactivity of the product, small particle size which allows a slow release of the oil, minimum volatilization, less labor intense and the most important aspect of all is that this technology reduces human exposure to toxic compounds.

Recent Publications:

The results and experimental design obtained from this research was presented at the Almond Board Conference in Modesto, California in December 2007.