Control of *Varroa* mites in honey bees through the systemic application of essential oils.

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THE PROBLEM AND ITS SIGNIFICANCE:

Almonds are California's largest acreage tree crop, with the Central Valley accounting for nearly 100% of the U.S. domestic production (Connell 2002). Most commercial almond cultivars require pollination for nut set. Honey bee colonies are introduced into orchards to supply pollinators. Colonies should contain large populations of adult bees and brood for effective pollination. Recently, *Varroa destructor,* a parasitic mite of honey bees *Apis mellifera* (L.), has had a catastrophic effect on populations of managed and feral honey bee colonies. Beekeepers and the beekeeping industry worldwide have reported epidemic losses of managed bee colonies ranging from 25 to 80% (Finely et al. 1996; Tew, personal communication).

Since 1988, an EPA-registered plastic strip impregnated with the contact pyrethroid pesticide, fluvalinate (Apistan® strips) has been available to control *Varroa*. However, in the last few years, reports of fluvalinate-resistant *Varroa* in the U.S. have become common (Elzen et al. 1998, 1999a & b). The organophosphate coumaphos (CheckMite+®) recently has been used to control fluvalinate-resistant *Varroa*, but mites resistant to coumaphos are already evident (Elzen & Westervelt 2002). Coumaphos is at best, a short-term solution. According to EPA, coumaphos may exceed its chronic aggregate risk due to levels found in groundwater (EPA, 2000). There are an increasing number of sites across the U.S. where Varroa appears to be resistant to both coumaphos and fluvalinate (Elzen et al. 2001; Elzen & Westervelt 2002). Furthermore, fluvalinate and coumaphos are lipophyllic and can contaminate honey, beeswax and other hive products (Wallner 1999).

Beekeepers need immediate relief from *Varroa* infestations and the development of new control methods is crucial. Ethereal plant oils, also known as essential oils, can control bee mites (Calderone et al. 1997; Colin 1990; Imdorf et al. 1999; Sammataro et al. 1998). However, an effective delivery system for the oils has not been developed. Recently, a commercial essential oil product for *Varroa* control has become available in the United States (Apivar - thymol and camphor based). Apivar is designed to create a plume of oil vapors that kill the Varroa on adult bees. The major drawback of vapor-phase oil products is the high variation in oil volatility under different environmental conditions. Consequently, mite control with a vapor phase delivery system is unpredictable.

PROJECT GOALS

Research Abstract

The focus of this project is to determine if it is possible to interrupt Varroa mite reproduction by introducing small amounts of highly emulsified essential oils into the honey bee hemolymph through a delivery system specifically developed to carry the oils to the gut of the larvae via the adults. This technique will allow us to determine whether the oils will disrupt mite reproduction through cessation in feeding or through acute mite toxicity.

The goal of this project is to limit *Varroa* mite reproduction by developing a means to deliver essential oils to honey bee larvae through a systemic (feeding) delivery system. Adult bees will be fed our liquid protein diet with added essential oils. The diet will be distributed throughout the colony by food exchange among adult bees (trophalaxis) and fed to developing larvae by nurse bees. Larvae fed the diet will receive systemic doses of the oils. In addition to the liquid protein diet, we plan to investigate several different carriers for the essential oils, including pollen patties, and soy supplement patties.

Results in the past year demonstrated that the essential oils are better delivered to the larvae when they are incorporated into a protein-lipid diet as opposed to straight sugar syrup. When the oils were emulsified in the newly developed liquid protein diet, mite reproduction/cell dropped below1.0, indicating that the mites were unable to effectively reproduce, however, when the same oils were emulsified in sugar syrup the same level of control was not observed. We have identified three oil formulations that work the best.

OBJECTIVES

The specific objectives of the project are:

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.**

METHODS AND RESULTS

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.

Results

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.

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. Standards were collected with a brief exposure to the oil standards so as to not overload the fiber.

Results

All test colonies consumed greater than 250 ml of diet oil mix each week indicating that the mix was getting into the bee. Utilizing the SPME fiber as a volatile sampling method, the GC analysis revealed the oil in larval and prepupal samples.

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

Carrier Compatibility

The liquid protein honey bee diet developed by our team was reformulated to improve the hydrocolloid suspension and to reduce bacterial growth and contamination. 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. Upcoming gas chromatograph analysis will determine if essential oil in the hemolymph is mode of action in these trials. 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 (#3) 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 (Table 1). 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

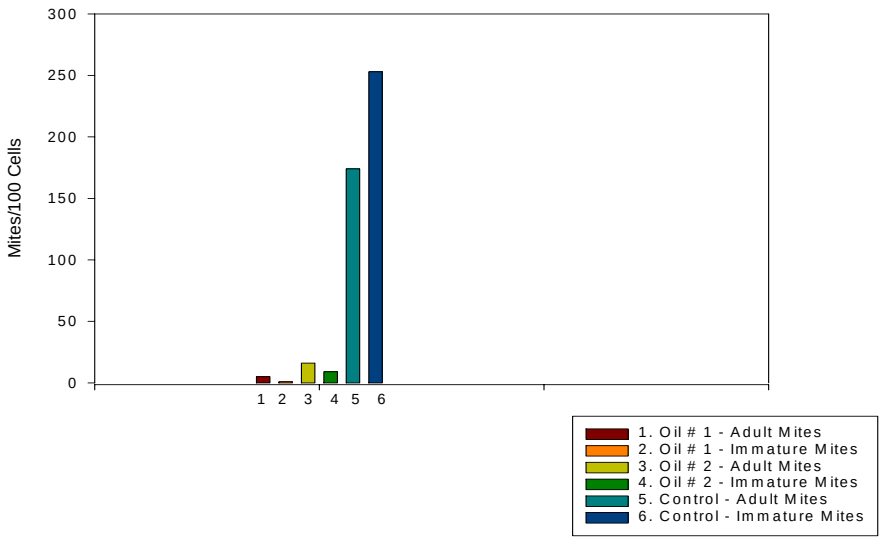


Table 1. Clearly demonstrates the ability of the oils in the diet to reduce mite invasion and mite reproduction in the colony cells.

Table 2

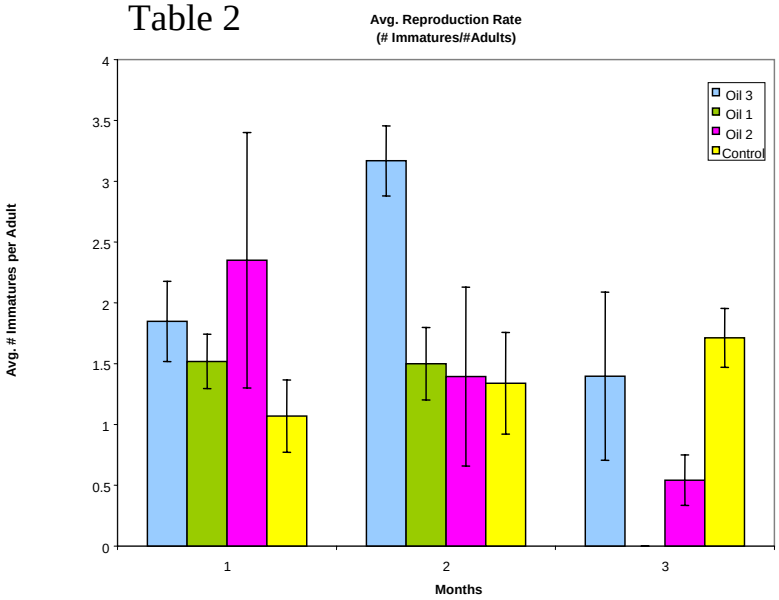
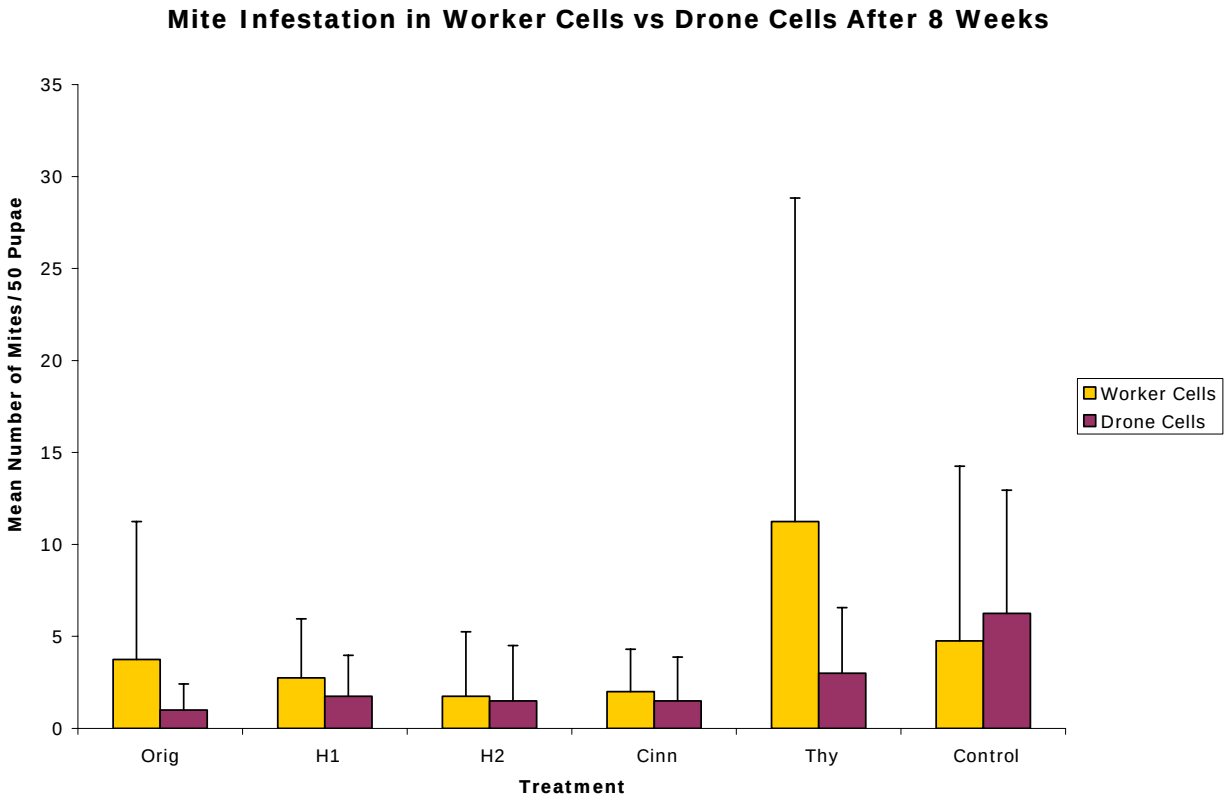


Table 2 demonstrates that mite reproduction decreased over time, and after three months, mite reproduction dropped below a ratio of 1.0 mites per infested cell. At a level below 1.0 mite per infested cell, the mite populations will soon crash due to a lack of replacements.

Table 3



Normally mites will invade the drone cells before invading the worker cells due to brood pheromone attractants. Table 3 demonstrates that the mites invaded the worker cells in greater numbers than they invaded drone cells. Only the controls showed more mites in drone cells than worker cells. This could be due to a disruption in host recognition due to the smell of the oils or the drones being of larger size received more of the treated diet and consequently had a stronger odor of the oils. An experiment will be designed around this observation in the coming year.