

Dear National Honey Board,

On behalf of my colleague Dr. Mike Scharf, I am writing to you to share with you the results from our research project that you funded in 2009/2010. The title of the project was “Determining if varroa mites (*Varroa destructor*) and pesticides interact synergistically to harm developing honey bees (*Apis mellifera*).” We have completed the project and, as you will see, have found exciting results.

To facilitate sharing the results with you, we include 3 abstracts from the three separate, yet related, projects we conducted. All three contributed significantly to our understanding of potential synergisms between varroa and pesticides.

We did experience a number of complications in our study. In our original proposal, we outlined three experimental objectives that we felt would address our purpose adequately. They were:

- (1) Determine if 5<sup>th</sup> instar bee larvae reared on a pesticide-laced diet are more/less attractive to foundress varroa than 5<sup>th</sup> instar bee larvae reared on a pesticide-free diet. ***Phase I: varroa choice tests.***
- (2) Determine if pupae reared on a pesticide-laced diet experience higher mortality than control pupae when hosting varroa while developing. ***Phase II: pupal mortality.***
- (3) Compare/contrast the reproductive capacity (number of offspring produced) of varroa parasitizing control pupae and pupae reared on a pesticide-laced diet. ***Phase III: varroa fecundity.***

We were able to complete objective 1 as stated in the original proposal (see Abstract 2), but only after conducting another study to determine what doses of each of 9 pesticides warranted testing. As such, we had to include an “unplanned” study in the overall design. I include our results from that study in Abstract 1 as the study was instrumental to the success of our other projects and because we used NHB funding for the project.

Objectives 2 and 3 were considerably more difficult to accomplish than we had anticipated. In short, it is difficult to rear honey bees *in vitro*, especially through the pupal stage. Furthermore, our original design called for us to allow varroa to reproduce on pesticide stressed pupae *in vitro*. After repeated attempts, we could not get the varroa to reproduce on bee pupae *in vitro*. Though seemingly problematic, this led to our development of a third project which, after completed, yielded better data than our original planned project.

In the new project (outlined in Abstract 3), we teamed with Dr. Ales Gregorc (post doctoral research fellow in my lab) and Dr. Jay Evans (USDA-ARS, Beltsville, MD) to study how varroa and pesticides interact to affect the expression of genes in developing pupae. As such, we were able to accomplish our overall project goal in a manner not foreseen originally. At the end of the day, I think you will

agree that this new project produced results that were more exciting than what could have been produced by our original proposed project, all while accomplishing the overall goal of the project anyway.

Not to belabor the point, the abstracts take you linearly through work funded by the NHB, from what doses of pesticides affect developing larvae (Abstract 1), to how varroa respond to treated larvae (Abstract 2), and concluding with how pesticides and varroa interact to affect gene expression in bee pupae (Abstract 3). Your support of these three projects was indispensable and is greatly appreciated.

Please contact me should you have any questions about this report. Thank you again for supporting this research effort. I hope that we can work together again in the future.

Sincerely,

Jamie Ellis  
Assistant Professor of Entomology  
Honey Bee Research and Extension Laboratory  
Department of Entomology and Nematology  
University of Florida  
352 273 3924  
jdellis@ufl.edu

## **Abstract 1: Determining LC<sub>50</sub> values for pesticides affecting larval honey bees**

The overall objective of this research project was to determine the effects of pesticides on immature honey bees. More specifically, we determined the LC<sub>50</sub> values of 5 insecticides (chlorpyrifos, imidacloprid, amitraz, fluvalinate, coumaphos), 2 fungicides (mycobutanil, chlorothalonil), and 2 herbicides (glyphosate, simazine) on developing bee larvae. To do this, we manipulated honey bee colonies to produce same age larvae, grafted these larvae into 96-well tissue culture plates, treated the larvae with one of the test pesticides at various doses, and reared the larvae to defecation. The larvae were treated with one of 6 pesticide concentrations at 132 h, 156 h, 180 h, and 204 h during their development. Two controls were included in the study, one of acetone only in the diet and a second with no acetone or pesticide application whatsoever. The replicate schedule was 9 test pesticides × 8 treatments (6 pesticide doses and 2 controls) × 8 queen sources × 8 larvae/treatment. Mycobutanil and glyphosate appeared virtually non-toxic to developing larvae at the concentrations tested (0 - 400 ppm). Simazine and amitraz were slightly toxic to developing larvae (LC<sub>50</sub> = 3163 ppm and 2304 ppm respectively) while imidacloprid and chlorothalonil were more toxic to developing larvae (LC<sub>50</sub> = 706 ppm and 351 ppm respectively). Chlorpyrifos was the most toxic compound tested with a LC<sub>50</sub> of 1.6 ppm. Though fluvalinate and coumaphos clearly affected developing larvae, the departure of their concentration-response curves from an expected probit model suggests the development of resistance to these pesticides by developing larvae; an effect probably elicited by long-term selection with both pesticides in association with efforts to control varroa mites in honey bee colonies.

## **Abstract 2: Does a history of pesticide exposure affect larval honey bee attractiveness to varroa mites?**

In this study, we tested how varroa respond to pesticide treated larvae. Specifically, we determined if larvae fed a diet containing either high or low doses of one of 5 insecticides (chlorpyrifos, imidacloprid, amitraz, fluvalinate, coumaphos), 2 fungicides (mycobutanil, chlorothalonil), or 2 herbicides (glyphosate, simazine) were more or less attractive to varroa than larvae fed a control diet or a diet containing acetone. To do this, we constructed choice test arenas for varroa using small Petri dishes (~5 × 2 cm, diameter × depth), each containing 4 plastic cell cups (~0.5 × 1 cm, diameter × depth). We flooded the Petri dish with beeswax to secure the cell cups. For each of the pesticides listed, we reared 4 larvae fed either (1) a diet with a “high” dose of pesticide, (2) a diet with a “low” dose of pesticide, (3) a control diet, or (4) diet with acetone. The four larvae were put randomly into the 4 cells cups within the Petri dish. Following this, we introduced 10 varroa mites into the Petri dish. Twenty four hours later, we counted the number of varroa on each prepupa. This procedure was replicated 10 times for each pesticide. Neither bee larvae nor varroa were used more than once. Upon analysis, the data suggest that exposure to pesticides while feeding did not affect larval attractiveness to phoretic varroa. Varroa entered cell cups containing treated larvae indiscriminately. In conclusion, our data suggest that pesticide exposure history does not predispose treated larvae to being parasitized by varroa.

### **Abstract 3: Do varroa mites and pesticides synergize to affect honey bee pupae?**

Two sets of honey bee larvae reared in an incubator were fed a diet for two days *ad libitum* containing either 0.8 ppm chlorpyrifos, 200 ppm imidacloprid, 200 ppm amitraz, 100 ppm fluvalinate, 50 ppm coumaphos, 200 ppm mycobutanil, 200 ppm chlorothalonil, 200 ppm glyphosate, 200 ppm simazine, or diet alone. Pre-pupae resulting from the second set then were exposed to two varroa mites for two days. All prepupae were screened for the expression of 46 candidate genes implicated in cellular and immune response, development, and presence of pathogen genes, along with two controls for transcript abundance (encoding actin and RPS5) using real-time PCR reactions. Both pesticide exposure and/or varroa mite parasitism on honey bee larvae led to changes in gene expression, specific for genes that were upregulated, down regulated or neutral. Transcript levels for PGRPSC 4300, a pathogen recognition gene, increased in larvae exposed to varroa mites ( $P < 0.001$ ) and were not changed across pesticide treated larvae. Similarly, DWV transcripts increased substantially with exposure to varroa mites ( $P < 0.001$ ), but not with exposure to pesticides. Transcript levels for PPOact, an immune end product, differed across pesticide treated larvae ( $P < 0.001$ ). Significantly higher expression of RNA for this gene was found in imidacloprid, amitraz, mycobutanil, and chlorothalonil treated larvae. Transcript levels for the storage protein AmHex70 decreased significantly after pesticide or varroa mite exposure ( $P < 0.01$ ). Down regulation effects also were seen for the gene encoding a cytochrome p450 member (CYP4g11), where the effect of mites was significant ( $P < 0.01$ ) in comparison to non infested larvae. The effect of chlorothalonil and varroa mites also was significant in downregulation of transcripts for this CYP. In conclusion, our data show that there were significant effects of varroa exposure and pesticide exposure on numerous and variable genes, though no synergistic effects between the two were noticed.