Investigation of the Impact of Pesticides and Adjuvants on Bee Health, and Development

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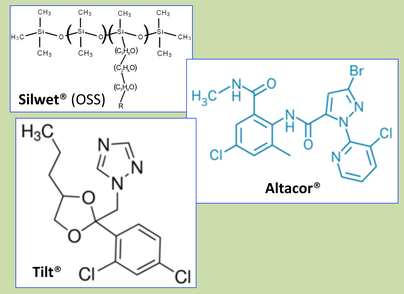


BACKGROUND AND DISCUSSION:

Success in almonds depends upon pollination. For honey bees, four factors interact to cause losses: varroa parasitization, pathogens, lack of good nutrition (pollen availability) and finally pesticide exposure. Some of the factors that may be common to many crops are fungicides and adjuvants. In pollen collections, fungicides rank in the top pesticides detected in incoming pollen; besides these compounds, **adjuvants** may be an issue. The amount of organosilicones (OSS) used annually has been increasing since 2000, as illustrated by usage in California (Figure 1a). OSS adjuvants can be used up to 1-5% in tank mixes. Recommended usage for IPM ranges from 300 ppm to 5000 ppm in one spray. If used multiple times per year, the actual exposure to OSS is not known. Added research is needed to understand the impacts of pesticides on honey bee colony health. Potentially the interaction of pathogen infections and pesticides could be altered when combined with adjuvants.

Pesticides have been demonstrated to impact pathogen infections in bees (Degrandi-Hoffman et al, 2015). The OSS adjuvants are themselves highly toxic with different forms varying in the level of toxicity (Fig. 1b) (Mullin et al, 2015; Chen, Fine, and Mullin, 2018). These adjuvants are of concern since OSS have bee detected in 60% of pollen samples (Chen and Mullin, 2013b). When used in *in vitro* rearing of honey bee larvae, viral exposure and OSS synergized to result in highly significant mortality at 40 ppm (J. Fine, Mullin, and Cox-Foster, 2017) with increased viral titers and depressed immunity.

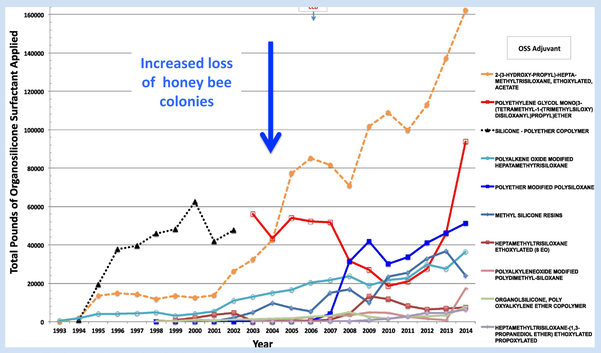
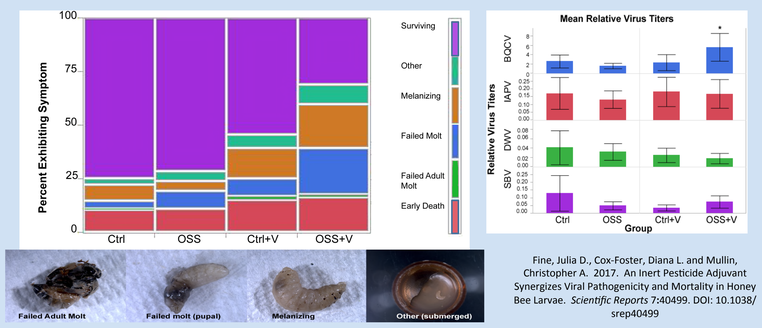


Fig. 1. OSS's differ in toxicity to bees, with increased use of most toxic OSS in CA since 2000. Data on OSS use from the California Pesticide Information Portal Project (CalPIP) in pesticide use databases. From: Chen J, Fine JD, Mullin CA. 2018. Are organosilicon surfactants safe for bees or humans? Sci Total Environ. 612:415-421.

In this research, we asked about the impacts of a fungicide/ insecticide mixture (Propiconazole (Tilt) at 150 ppb a.i. and chlorantraniliprole (Altacor) at 3 ppm a.i.) and a commonly used adjuvant (organosilicones (OSS) or Silwet, 40 ppb). The four treatments consisted of untreated, fungicide/insecticide-treated, organosilicone-treated, and fungicide/insecticide/ organosilicone-treated.



Fine, Julia D., Cox-Foster, Diana L. and Mullin, Christopher A. 2017. An Inert Pesticide Adjuvant Synergizes Viral Pathogenicity and Mortality in Honey Bee Larvae. *Scientific Reports* 7:40499. DOI: 10.1038/srep40499

Figure 2. Organosilicone adjuvant and viral exposure synergize in developing honey bee larvae to result in elevated Black Queen Cell Virus titers and larval death at pupation. These symptoms mirror those described by bee keepers following almond pollination.

METHODS:

1. Micro colonies were used to create colonies with similar pathogen loads and to test the "worst case scenario", since these colonies lack the resilience of a large workforce of a normal colony.
   a. Sister queens (same genotype/source) were used, and workers came from the same colonies
   b. Small hives that interlock were used and expanded as needed.
2. Colonies were fed treatments incorporated into UltraBee artificial pollen, and known amounts were given on regular basis. Unlimited sugar water (1:1) was provided
3. Samples and images were collected on regular basis. Samples were collected for pathogen analysis and frozen at -80°C. (Analysis is currently being done for viruses, fungal, microsporidia, and protozoan pathogens.)

OBJECTIVES:

1. **Confirm the impact of the fungicides/pesticide combination, organosilicone adjuvants, and combination on bee survival and reproduction, resolving the minimal colony size needed to alleviate the impacts.**
2. **Evaluate the impact of fungicides and organosilicones on overall pathology of bees and correlate with the colony level**

RESULTS AND DISCUSSION:

Microcolonies were initially established with equal number of workers, closed for 1 week, and then opened to forage. During this time the workers re-assorted themselves, resulting in 3 strengths (#workers/queen) levels. The colonies were randomly assigned to the treatments with each treatment having colonies with all three levels. Colonies were placed in an apiary with each treatment randomly placed in each row.

Figure 2. (left) View of apiary and microcolonies at mid-season; additional supers have been added onto some colonies as they expanded. (**right**) Layout of colonies and assignment of treatments (Green= Control, Yellow= OSS, Blue= Tilt and Alticor, Red= Tilt, Alticor, and OSS).

HOW DID THE PESTICIDE/ADJUVANT TREATMENTS AND INITIAL COLONY STRENGTH IMPACT THE COLONY SURVIVAL?

1. **Both initial colony size and chemical treatments significantly affected the growth of the colonies.**

Growth has been initially measured by number of frames with built comb. (The actual number of workers will be determined over time.)

Strong colonies at start of treatment expanded in all treatments; however, colonies given Tilt and Alticor expanded at a lower level as compared to the Control or OSS alone treatments.

Weak colonies died in all of the pesticide/adjuvant treatments during the summer; whereas, several weak colonies in the Control treatment group have gone into the winter.

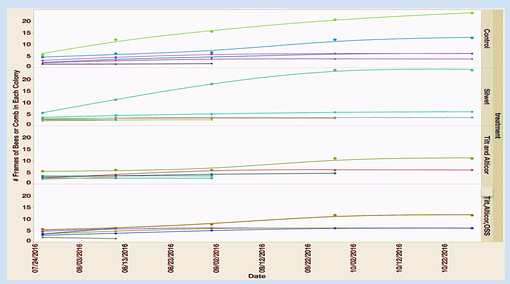


Figure 3. Growth of colonies, as measured by frames having comb over the season, for the 4 treatments. Where a line ends, the colony died. Size of circle represents the initial size of the colony. Death of colony was determined when the queen was lost. Parametric Survival Fit was tested, and both initial size and treatment were significant predictors.

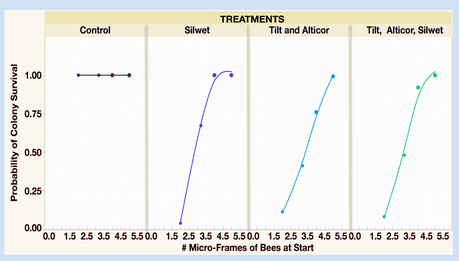


Figure 4. Survival probabilities predicted by the following model.

Parametric Survival Fit

Distribution	AICc	BIC	
Frechet	128.86851	127.83839	Best

Whole Model Test

ChiSquare	DF	Prob>Chisq
25.9522	7	0.0005*

Effect Likelihood Ratio Tests

Source	Nparm	DF	L-R ChiSquare	Prob>ChiSq
Treatment	3	3	9.76934124	0.0206*
Initial Size	1	1	23.8948274	<.0001*
Initial Size*Treatment	3	3	5.98747362	0.1122

HOW DID THE PESTICIDE/ADJUVANT TREATMENTS AND INITIAL COLONY STRENGTH IMPACT QUEENS AND COLONY BEHAVIOR?

Two types of major events occurred during the research: **queen loss (death)** and **swarming/absconding** by the colony. Queens were marked and wings clipped, permitting identification; status of queens and colonies were monitored twice weekly until the end of August, once weekly in September, and twice in October.

Surprisingly for the **swarming/absconding**, no queen cells or replacements were observed in any of the colonies. The queens and workers crawled away from their hives and were found about 10 meters away as a small cluster. Later in the season, the source colonies were identified given the unique marks/wing clips on each queen. Samples have been saved for all queens and workers from swarms or for workers from colonies with dead/lost queens.

Queen loss and swarming were both significantly associated with the initial size of the colony (strength) and with a trend for association with treatments. ***The results suggest that small colonies can survive and continue to grow; however, with chemical treatment, these colonies were apt to be lost, due to queen loss or swarming behavior.***

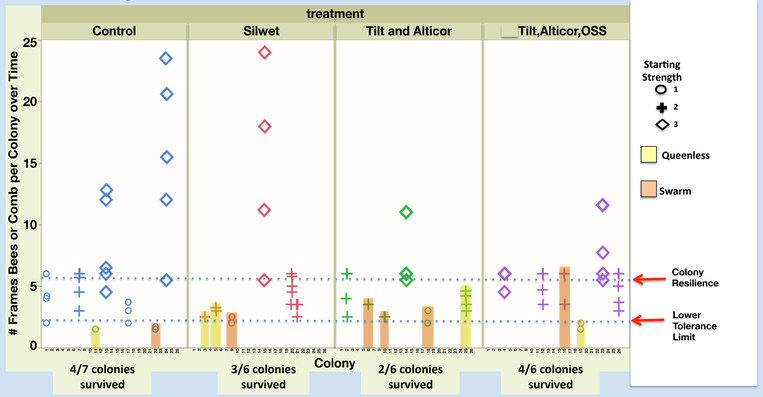
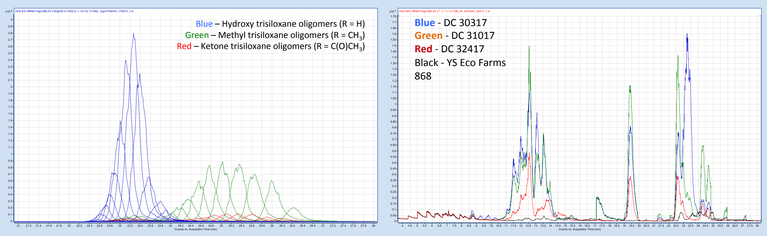


Figure 5. Queen loss and swarming are affected by starting strength and impacted by treatment. Parametric survival analysis, Weibull distribution, with censor. Whole Model Test (ChiSquare =21.0382, DF 11, Prob>Chisq 0.0330)(Effect summary: Starting strength p=0.00010, Treatment p=0.05534, Treatment X strength p=0.99811)

HOW DID THE TREATMENTS AND INITIAL COLONY STRENGTH IMPACT PATHOGEN LEVELS?

Analyses are still being performed, but initial results indicate that the colonies were infected with at least 4 viruses, Nosema, and chalkbrood. In the limited number of hives examined, the virus load for DWV and BQCV increased over time in the hives given the pesticides but not in the control hives. More samples need to be examined.

Development of OSS assays: In collaboration with Dr. Bill Doucette (USU), we have developed the ability to assay OSS in pollen and bee samples. In 3 samples of pollen from almonds, we have found OSS and metabolites at more than 300 ppb and in bees, at 60 ppb.



Continuing Research: We are currently asking what concentrations of OSS bees encounter in almonds (in collaboration with Joel Siegel, USDA-ARS), and how other forms of OSS impact bee health. We are asking how gene expression is changing in response to the chemicals.

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