Investigation of the Impact of Fungicides and Adjuvants on Bee Health, Behavior, and Development

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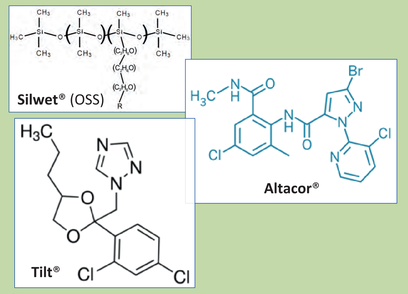
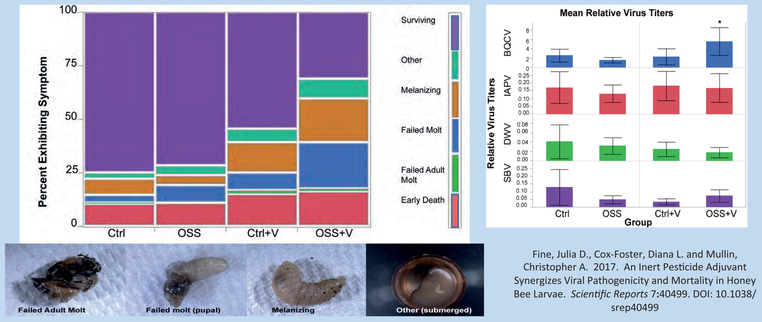


BACKGROUND AND DISCUSSION:

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) applied to almonds was approximately 10% the poundage of all active ingredients applied as reported in the Summary of Pesticide Use Report Data by the California Department of Pesticide Regulation for 2013. Added research is needed to understand the impacts of pesticides on bee behavior, health, and development when fed to colonies. Potentially the interaction of pathogen infections and fungicides could be altered when combined with adjuvants.

Fungicides via the pollen provisions do result in elevated pathogen levels (*Nosema* and viruses) and impair the food digestion/ absorption of honey bees (Degrandi-Hoffman et al, 2015). Much higher toxicity occurs when honey bees are fed directly on related OSS in 50% sucrose, with oral LC_{50}s for OSS surfactants below 10 ppm (Mullin et al, 2015) (J Chen *et al*., unpublished). These adjuvants are of concern since OSS can be detected in 60% of pollen samples, up to 39 ppb (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and OSS synergized to result in highly significant mortality at 30 ppb or lower (J. Fine, Mullin, and Cox-Foster, unpublished) with increased viral titers and depressed immunity.



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 1. 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.

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.

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 obtained from Kona Queens, and workers came from the same colonies (Darren Cox, Logan, UT)
   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, given that it was a dry season.
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 FOR CURRENT YEAR:

1. **Evaluate impact of fungicides, organosilicone adjuvants, and combination on bee survival and reproduction**
2. **Determine if fungicide and organosilicone elevate viral titers in individual bees to alter behavior (ongoing research)**
3. **Evaluate the impact of fungicides and organosilicones on overall pathology of bees (ongoing research)**

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.

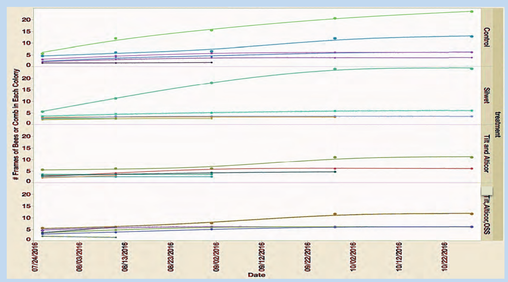


Figure 3. Growth of colonies, as measured by frames having comb over the season, for the 4 treatments. Where a line ends, the colony died. Size of circle represents the initial size of the colony. Death of colony was determined when the queen was lost. Parametric Survival Fit was tested, and both initial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

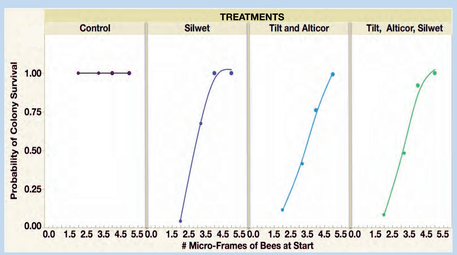


Figure 4. Survival probabilities predicted by the following model.

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.***

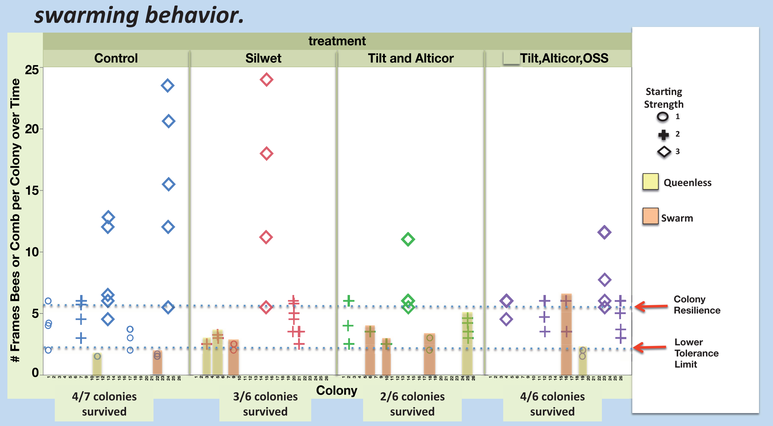


Figure 5. Queen loss and swarming are affected by starting strength and impacted by treatment. Parametric survival analysis, Weibull distribution, with censor. Whole Model Test (ChiSquare =21.0382, DF 11, Prob>Chisq 0.0330)(Effect summary: Starting strength p=0.00010, Treatment p=0.05534, Treatment X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

HOW DID THE PESTICIDE/ADJUVANT TREATMENTS IMPACT THE LEARNING OF THE BEES?

Bees were marked according to treatment; trespassers were collected from inside each colony over time. ***Among the treatments, marked bees from F/I and OSS were more apt to be found in other colonies besides their own following consumption of the treatments.***

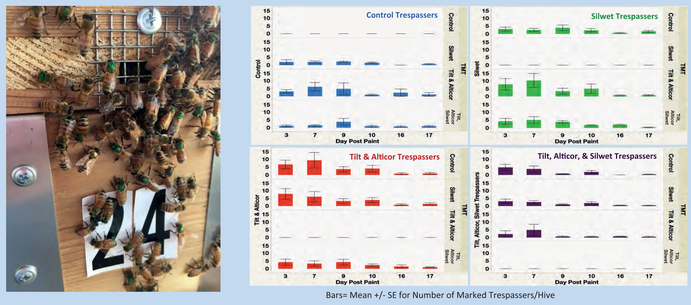


Figure 6. To assess impacts of the treatments on bee learning, bees from each colony were marked according to treatment; trespassers (bees with the wrong color) were collected from inside each colony over time. These bees will be assayed to determine if the viruses are at higher levels in their brains as compared to marked bees belonging to the right hive.

Continuing Research: We are currently assessing the impacts of the treatments on the pathogen levels (viruses, microsporidia, fungi, and protozoa) in the bees (eggs, larvae, workers, and swarm queens) during the summer. Colonies will be evaluated for winter survival.

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