AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2014 – 2016

TITLE: Effect of Nano-Formulated Chlorothalonil Fungicide Products on Honey Bee Development

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SUMMARY:
Beekeepers have reported effects on developing honey bee brood during almond pollination (Fig 1), which they attribute to applications of agrochemicals. Our preliminary work investigating various fungicides indicated that iprodione and chlorothalonil may affect developing brood. Often toxicological studies are performed with the active ingredient alone, and we have found that the pesticides associated with these active ingredients are formulated as particles. Some of these particles range into the nanoscale, which may be of significance in their regulation by EPA. We hypothesize that these pesticide particles may affect exposure to individual bees and transport and accumulation within colony materials. Under this proposal, we had intended to perform a semi-field trial exposing bee colonies to chlorothalonil, comparing it to Bravo WeatherStik, a nanobased pesticide formulation. During almond bloom in early 2014, a significant event affecting more than 80,000 bee hives induced us to examine the products that were most closely associated with this devastating bee kill. Using the protocols intended for chlorothalonil / Bravo Weatherstik, we investigated whether the insecticide Tourismo or its active ingredients (Flubendiamide and Buprofezin), applied with or without the fungicide Protocol (propiconazole and thiophanate-methyl) had affects resembling those observed during the 2014 almond bloom.

Figure 1. *During almond pollination, multiple agrochemicals are applied while bees are flying.*

Figure 2. *Students work in pairs to evaluate colony health, frame by frame.*

OBJECTIVES:
Our long term objective has been to determine what pesticides, if any, have caused periodic problems for beekeepers during almond bloom. Our original objective under this proposal was to evaluate long-term effects of chlorothalonil on honey bee colony health. When new

evidence became available, we compared flubendiamide and buprofezin with their product formulation, Tourismo.

PROCEDURES:

Bees and Bee Hives. We obtained nucs from a local beekeeper reared under similar conditions, and housed them in them in full deep hive boxes with new plastic frames. We evaluated each hive in order to group them by strength.

Pollen preparation. We spiked pesticide-free pollen (Hummingbird Wholesale) with field relevant concentrations of 1. Flubendaminde and buprofezin, 2. Tourismo (a nanoformualted product of flubendiamde and buprofzein), 3. Tourismo plus Protocol (a nanoformulated product of thiophanate-methyl and propiconazole), or 4. Control. Tourismo and Protocol particles can be seen in figure 3. We added sugar syrup to the pollen, and fed it to hives as pollen patties.

Effects on honey bees. 5 undergraduates were employed to evaluate colonies, working in pairs (Fig. 2). Colony evaluations were distributed throughout the week, so that each colony was evaluated weekly, and all conditions were paired with controls on a given day. We quantified eggs, larvae, capped brood, adult bees, presence of queen, and stores of nectar, honey, and pollen. Colonies were evaluated in detail weekly for 4 weeks, in addition to 2 weeks before pesticide introduction. High outdoor temperatures during one week prompted a break in evaluations.

Additional studies. Multiple samples of various materials were collected in order to follow through with any affects observed. We may also defer the analysis of these samples until additional funding is acquired. These samples may enable us to compare the persistence of the active ingredients to the formulated products used in our study in hive materials.

Statistical Analysis of Semi-Field Study

7 replicate colonies of each treatment were performed, and statistical analysis was performed using GraphPad Prism 5.

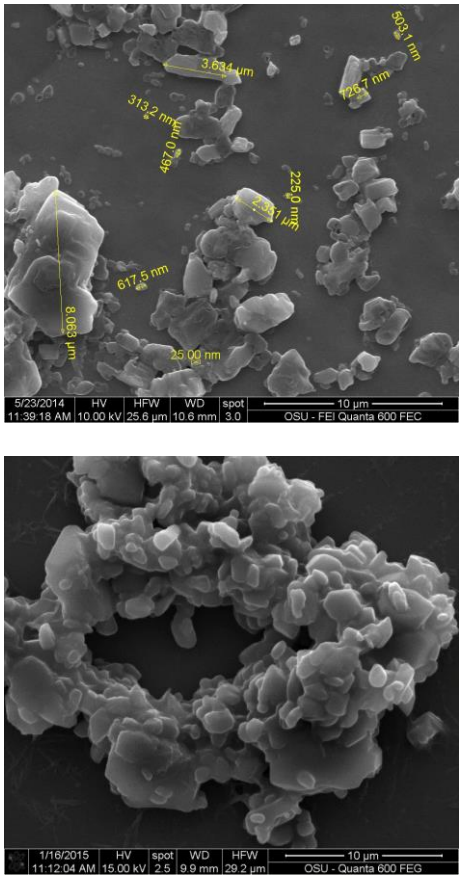

Figure 3. *Scanning electron microscopy of the nanobased pesticide Tourismo (top). Tourismo's active ingredients are flubendiamide and buprofezin. Below, SEM of Protocol, a fungicide composed of thiophanate-methyl and propiconazole.*

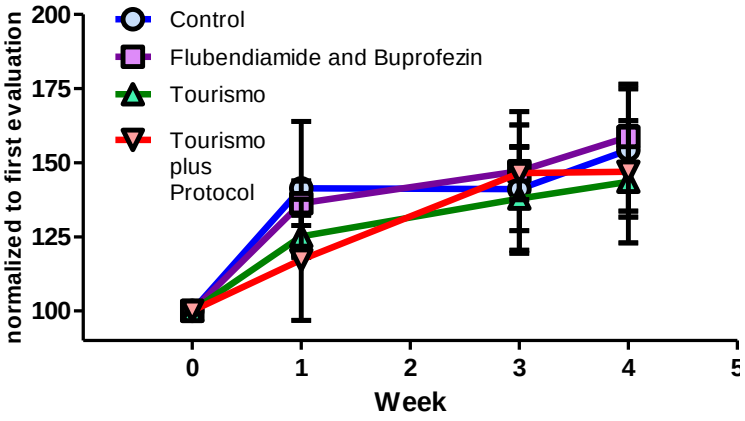

Figure 4. No *significant difference was observed when colonies were evaluated longitudinally, based on eggs, larvae, and capped brood.*

Table 1: Interactions of various particles with bees

Particle classification	Particle examples	Diameter Range	Effects on Bees
Nano (definition varies) PM0.1 <100 nm ultrafine particles	Nanosilver	1.5-5 nm	Decreased Nosema spores, variable effects on longevity
	Diesel exhaust	7.5 - 1000 nm	Affects learning and stress response, degrades floral odors.
	Nanopesticide particles approximate lower range	≈50 nm-	This study
	Fugitive dust from seed planting	230 nm - 32 µm	Associated with bee mortality
PM2.5 <2500 nm fine particles	Nanopesticide particles approximate upper range	≈1-10 µm	This study
PM10 <10,000 nm coarse particles	Pollen	6-100 µm	
	Microencapsulated methyl parathion (PENNCAP-M)	30-50 µm	Colony mortality, storage in pollen

Characterization of Safari and Protocol pesticide particles. Scanning Electron Microscopy was used to characterize the size and morphology of pesticide formulations used in this study.

SIGNIFICANT ACCOMPLISHMENTS:

Results: A significant difference in treatments was not observed (Fig 4). Due to biological variability between honey bee colonies, it can generally be difficult to distinguish subtle treatment affects. However, given the significant effects observed during 2014 almond pollination, and the decided lack of effect we observed, we are confident in asserting that these pesticide products were not responsible for the 2014 bee kill, or periodic problems for bees observed in almonds.

Results of this research was presented at multiple conferences:

Louisa A. Hooven.
Bees and Nanopesticides: Can Nanotechnology Based Formulations Affect Pesticide Exposure to Honey Bees?
Sustainable Nanotechnology 2015 Conference, Portland, Oregon

L. Hooven, J. Son, R. Sagili, and S. Harper
Do Nanotechnology Based Formulations Increase Pesticide Exposure to Honey Bees?
2015 Apimondia International Apicultural Congress, Daejeon, Korea

L. Hooven, J. Son, and S. Harper
Bees and Nanotechnology Based Pesticides
2015 Nanoscale Science & Engineering for Agriculture & Food Systems
Gordon Research Conference, Waltham, MA

L. Hooven, J. Son, R. Sagili, and S. Harper
Bees and Nanopesticides
2015 America Bee Research Conference, Tucson, AZ

L. Hooven, J. Son, and S. Harper
Do nanotechnology-based pesticide products increase risk of toxicity to honey bees compared to cognate active ingredients?
2014 Society of Environmental Toxicology and Chemistry, Vancouver, BC

BENEFITS & IMPACT:
Until the cause of the 2014 bee kill is determined, it can and likely will occur again. Our results eliminate one potential cause. Although the pesticides we experimented with are unlikely to be involved, multiple particle types affect bee health (Table 1). According to the EPA, which had access to additional analyses from multiple sources, a tankmix of Intrepid (methoxyfenozide) and Rovral (iprodione) may have been involved in the 2014 bee kill. We are already examining how these particulate formulations may interact with each other.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:
A renewal of a USDA grant was submitted, but only 11% of grants were funded, and ours was not among them.

FUTURE FUNDING POSSIBILITIES:
Our proposal will be resubmitted to USDA in 2016. Proposals may also be submitted to the California Almond Board, and other commodity groups.