

Purdue University  
Department of Entomology  
Undergraduate Capstone  
Project Summary

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Project Title:

Effects of varying neonicotinoid concentrations on honey bee mortality in a semi-field environment

**Project Summary:**

Populations of *Apis mellifera*, the European honey bee, have been declining annually in the United States by between 29%-36% (Pilatic 2012). This year, national losses have been estimated to be as high as 50% (unpublished data). Honey bee researchers believe that this population loss is likely due to the buildup of many factors, including varroa mites, habitat loss, viral and protozoan pathogens, and the extensive use of pesticides in large-scale agriculture. Neonicotinoids are among the most widely used pesticides in agriculture, and significant research effort has focused on them.

Neonicotinoids are a relatively new class of pesticides used to treat 94% of all corn grown in the United States (Krupke 2012). They are also used for many other field crops including cotton, sorghum, soybeans, and sugar beets, totaling 147 million acres (“Our Commitment to Bee Health” 2013). They can be applied as a soil drench or more commonly as a seed treatment. This class of pesticides is highly water-soluble and becomes systemic within the plant.

Two neonicotinoids commonly used on large acreage crops include thiamethoxam and clothianidin, known as Cruiser and Poncho, respectively. Clothianidin is extremely toxic to bees. It has an oral LD<sub>50</sub> of 2.8 ng per bee and a contact LD<sub>50</sub> between 22-44 ng per bee (Laurino 2011). Each kernel of treated corn has between 0.25 and 1.25 mg of clothianidin coating it; so the amount of chemical contained on the seed coat of one kernel of corn is enough to kill thousands of bees. However, the LD<sub>50</sub> does not account for sub lethal effects that clothianidin causes in honey bees. It is necessary to understand these sub lethal effects because the accumulation of these effects will impact bee mortality over time and may result in increased national population loss.

The goal of this study was to gain a more comprehensive understanding of clothianidin’s effects on honey bee colonies by analyzing its impact on multiple aspects of the hive including worker bee longevity, presence of virus infection in the colony (Deformed Wing Virus and others), overwintering success, and persistence of neonicotinoids in the comb. The study reported here focuses on the effects that dietary exposure to clothianidin has on worker bee longevity. This experiment is unique in that it examines the effects of clothianidin in a semi-field environment, thus eliminating many confounding factors that would exist in a pure field study analyzing the same effects.

## **Materials and Methods:**

The experiment was conducted in a large high tunnel, which excluded the colonies from the outside environment. The tunnel was eight meters wide, fifteen meters long, and four meters high. There were three treatments used with four replications of each treatment. The three treatments included untreated (control) and two concentrations of clothianidin. The concentrations were based upon clothianidin concentrations found in studies of honey bees and colony contents sampled during 2010 and 2011 at various locations in northwestern Indiana. (Krupke et. al, 2012). The tent was divided into three sections using plastic mesh screening to separate each of the treatments. Twelve nucleus colonies were used (n = four per treatment).

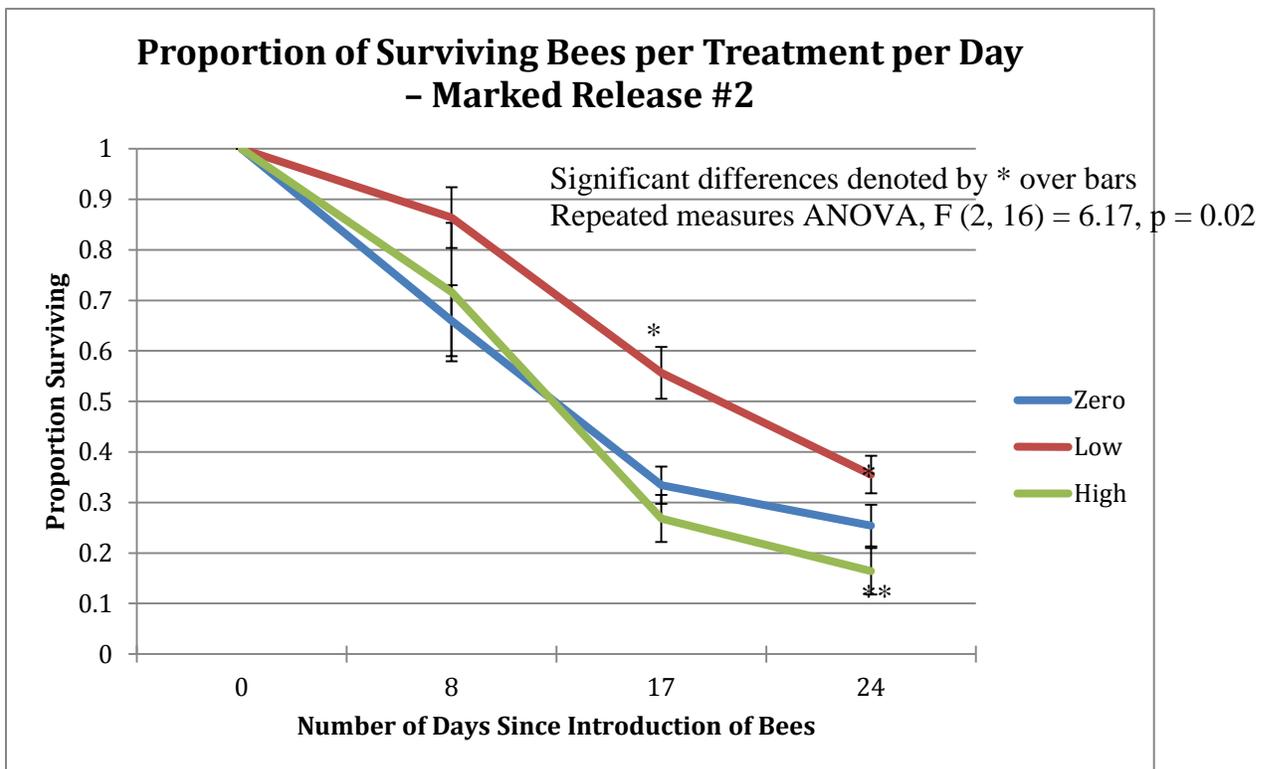
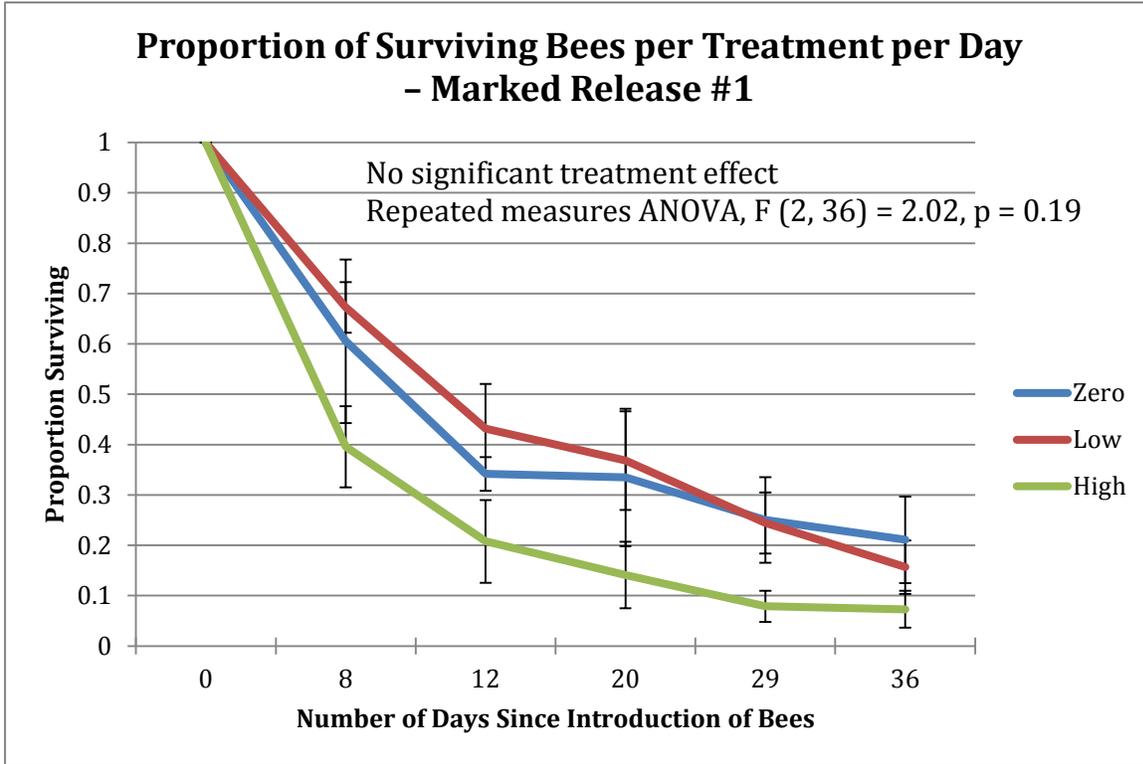
The nucleus colonies (nucs) each consisted of four clean frames and one feeder. The original population of bees consisted of two frames worth of bees, one frame of unhatched brood, and a queen reared at the Purdue honey bee laboratory. The queens used in the nucs were sisters and had taken mating flights in which they mated with drones in the area before being introduced to the nucs. The colonies were visually assessed for strength (number of workers and brood) and assigned to a treatment prior to the experiment so that hives of varying strength were represented in each treatment.

The clothianidin treatments were administered using pollen patties, which were fed to each nucleus colony. The patty mixture for each treatment was made up of 414 grams of Ultra Bee pollen supplement, 295 grams of white sugar, and 250 mL of sugar syrup. The zero treatment patties did not contain any clothianidin. Low treatment patties had 8.1 mg of clothianidin per kg of patty. High treatment patties had 88 mg of clothianidin per kg of patty.

The effects of the patties on worker longevity were studied using two introductions of newly emerged worker bees. Capped, un-emerged cells containing pupae were pulled from multiple colonies at the Purdue bee lab 12-16 hours prior to marking and stored in an incubator at 25 degrees Celsius where they emerged from the cells. All newly emerged workers were shaken into a large plastic container before randomly placing them into smaller ones at individual marking stations. Twelve frames were used at separate times for each marked group. These two groups were named "Marked Release #1" and "Marked Release #2." Each group of bees was marked by a dot of paint on their abdomens. Marked Release #1 bees were marked on September 5<sup>th</sup>, 2012 and installed into the colonies on September 6<sup>th</sup>. Each nuc received 100 marked bees. The pollen patties were also added to the hives on the 6<sup>th</sup>. Marked Release #2 bees were marked and installed into the hives on September 20<sup>th</sup>. Each nuc received 140 marked bees. At that time, new pollen patties were added and any remains from the old ones were discarded. Each group of bees was allowed four to six days of adaptation to the hives before baseline survivorship numbers were recorded. On October 24<sup>th</sup>, the experiment was concluded and all colonies were removed from the tent. The general health of the colonies was monitored over the winter.

Once every week, pictures were taken of each of the frames. Smoke was used to calm the bees and to have them remain on the frames while the photos were taken. After the conclusion of the experiment, all the frames were classified by week and treatment. A grid was superimposed on each picture to decrease the errors of counting and to standardize the counting method. The grid was an eight-cell by seven-cell grid created in Microsoft Word and each document was zoomed to 200% before counting. The number of marked bees on each frame per date was recorded on a Microsoft Excel spreadsheet. Using the initial post-release count as a baseline, these numbers were used to calculate the survivorship proportions of the marked bees. The proportions of each treatment were averaged and these averages graphed.

Data:



**Results:**

In Marked Release #1, the overall trend of the graphs indicates that bees that received the high treatment pollen patties had greater mortality than the bees that received the low or zero treatments. However, no differences were significant statistically.

In Marked Release #2, the trends are similar, although the only points that are statistically significant are the last two intervals for the low treatment (day 17 and day 24), which indicates that bees with low treatment suffered significantly lower mortality than those subjected to the zero or high dose treatment.

One high treatment nucleus colony (rep #3) died in the late fall of 2012. The remaining eleven colonies died before January 1, 2013.

**Discussion:**

The counterintuitive results from this experiment demonstrate the difficulties of working with honey bees. Bees are highly mobile and exhibit complex behaviors that are highly mediated by the environment, which makes them difficult test subjects when they are unable to forage as they would normally.

The fact that all of the overwintering nucleus colonies died may be a function of the environment of a semi-field study (Blacqui re 2012). There are many reasons why a semi-field study may be harmful to honey bees including the proximity of the colonies to one another, the restricted diet of the bees, the environment inside of the tent, and other reasons that have not been considered.

There were pitfalls in the experiment, which may have affected the results. First, the tent was damaged a few times during the experiment by storms and wind. The wind tore the tent walls and allowed bees to get outside as well as ripped down dividers between the treatments. Some bee colonies were tipped over. Second, the paint used to mark the second group of bees was more difficult to see and less permanent than the paint used to mark the first group of bees. These bees were more difficult to spot in the photographs and the paint appeared to rub off over time. Finally, the photos were not randomly counted; they were counted in the order the pictures were taken. This method could have led to bias, as the person counting grew more accustomed to the work over time. In the future, the pictures should be numbered randomly and then assigned to the person counting.

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