Sub lethal effects of neonicitinoids on Megachile rotundata

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Intro

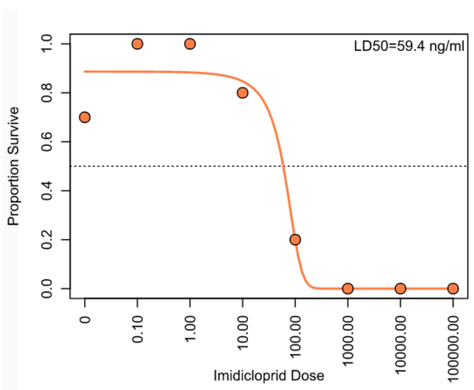
Neonicitinoids are heavily used in U.S. agriculture despite their effects on non-target organisms, such as *Megachile rotundata*. Previous literature has suggested that lethal dosages are well beyond what would be used in field applications, but little has been investigated regarding sub lethal effects and consequential effects on the next generation. Our project focused on determining high and low dosages through ingestion, rather than topical applications. We observed behavior and nest architectural between bees that had been exposed to a low dose of neonicitinoids with those that have had no exposure. We also compared upregulation of acetylcholinesterase enzymes of bees after exposure through color spectrometry using the methods of Ellman to determine how long neonicitinoids remain present in the system.

Hypotheses

- H_0: There will be no difference in lethality between concentrations.
- H_0: Low dosages of neonicitinoids would have no effect on nesting.
- H_0: Low dosages of neonicitinoids would show no upregulation of acetylcholinesterase enzymes.

The LD_{50} is lower than previously thought

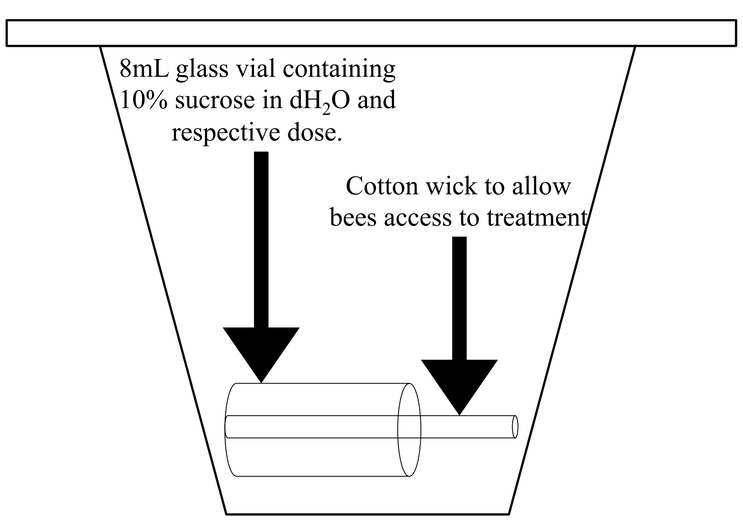
An LD_{50} protocol determines at which dose of the neonicitinoid, Imidocloprid, 50% of those immediately dosed experience lethal effects. This protocol helped determine which dose would be an appropriate "low dose" to use in prior methods.



Graph 1: The dosage in which 50% of those treated experience lethal effects (the LD_{50}) is at 59.4ng/ml.

Experimental design

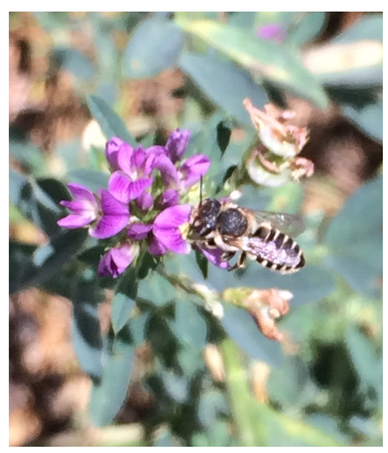
- 10 bees per treatment container
- Dosed continuously for 24 hours



Exposed bees did not nest

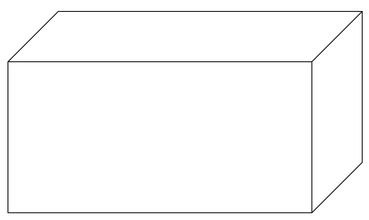
Two cages containing bees that either have been exposed or have not been exposed were set up next to each other in an alfalfa field, with buckwheat provided for leaf clippings. Behavior between the treatments was observed, and nests constructed by either treatments were compared.

Picture 1: Tents used in alfalfa field for collecting behavioral observations and nest measurments.



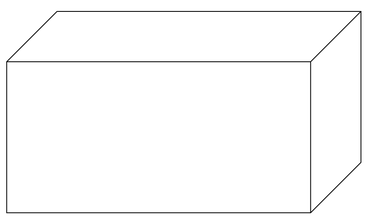
Picture 2: An Alfalfa Leaf Cutter Bee foraging on an alfalfa flower

Experimental design



Tent 1: Exposed Bees

- Released 50 females that had been exposed
- Released 25 males that have not been exposed



Tent 2: Unexposed Bees

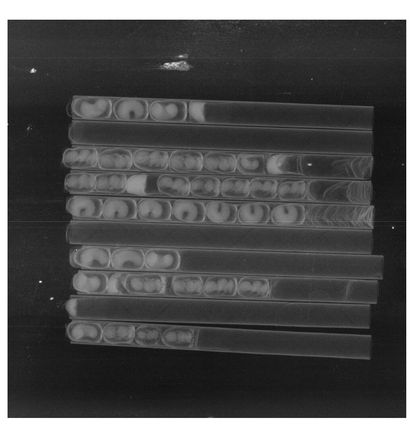
- Released 50 females that had not been exposed
- Released 25 males that have not been exposed

Replicate 1	# of Nests Build	# of Incomplete Nests	% Incomplete Nests
Control	**4**	**11**	**73.33%**
Exposed	**0**	**14**	**100%**

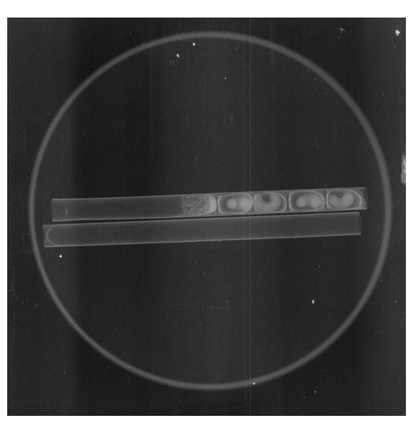
Table 1: Temporal replicate (1). 50 females and 25 males were released on June 20th, and the nest block remained in the field for 18 days.

Replicate 2	# of Nests Build	# of Incomplete Nests	% Incomplete Nests
Control	**18**	**8**	**30.77%**
Exposed	**0**	**4**	**100%**

Table 2: Temporal replicate (2). 30 females and 15 males were released on July 4th, and the nest block remained in the field for 18 days.



Picture 3: X-rayed photo of nests collected from temporal replicate (1) in the control cage

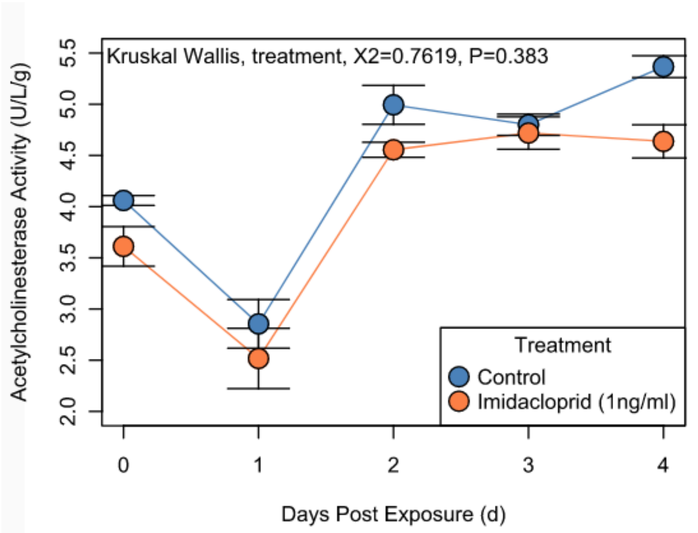


Picture 4: X-rayed photo of nests collected from temporal replicate (1) in the treated cage

Acetylcholinesterase was not upregulated

This protocol measured the concentration of upregulated acetylcholinesterase enzymes present in the organisms central nervous system. Bees were given a single low dose of 1.0ng/ml, determined in the LD_{50} conducted previously, and the upregulated enzymes were monitored throughout a 4 day period post exposure.

Picture 5: A plate of acetylcholinesterase enzymes after reacting to acetylhiolcholine iodide and Ellmans reagent for 35 minutes.
The top row were negative control samples.



Graph 2: Upregulation of Acetylcholinesterase throughout a 4 day period. The imidocloprid did not upregulate acetylcholinesterase enzymes.

Discussion

- While previous literature has suggested lethal dosages of neonicitinoids to much higher, we found dosages that are field relevant to have lethal effects when ingested.
- When exposed to minimal concentrations, bees will not construct completed nests.
- The enzymes may not have had up regulated due to being dosed only once, while in previous literature, they are continuously dosed.

Future Direction

- Add more replicates to LD_{50}
- Adjust Acetylcholinesterase assay to get readings more precise
- Repeat Acetylcholinesterase assay with continuous dosing
- Produce an LD_C
- Repeat field work without cages and instead with marked bees

Refrences

1. Boily, M., Sarrasin, B., Deblois, C., Aras, P., & Chagnon, M. (2013). Acetylcholinesterase in honey bees (Apis mellifera) exposed to neonicotinoids, atrazine and glyphosate: laboratory and field experiments. Environmental Science and Pollution Research, 20(8), 5603-5614

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