Toxicity Assessment of Glyphosate on Honeybee (*Apis mellifera*) Spermatozoa

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Introduction

- In 2015-2016, 44% of the managed honeybee colonies in the U.S. were lost due to Colony Collapse Disorder (CCD) (Traynor *et al*. 2016). Commonly used pesticides are one among the suspected causes for CCD.
- Effects of pesticides on honeybee reproductive physiology is understudied especially with respect to the drone bee.
- Drones are male honey bees. The queen bee receives semen from multiple drones once during her mating flight. The queen will store the semen in her spermatheca and use it throughout her life time which can last between 2-7 years (Winston, 1987).
- If pesticides have negative effects on the drone's spermatozoa this could lead to decline in reproductive success contributing to CCD.
- *N*-(phosphonomethyl)glycine (Glyphosate) is one of the most widely used herbicides in the U.S. and has been shown to have effects on behavior in the honeybee (Herbert *et al*. 2014). Nectar collected by the bees from plants that have been directly sprayed can carry between 2-32 mg/kg of glyphosate (Thompson *et al*. 2014).

Objective

- The purpose of this experiment is to test the effect of direct application of glyphosate on the spermatozoa collected from the honeybee drones.

Hypothesis

- Field concentrations of glyphosate negatively affect honey bee reproduction by reducing the number of viable spermatozoa in drones.

Methods & Materials

Sperm samples collected from drones USDA, Fargo or received from USDA, Baton Rouge, LA.

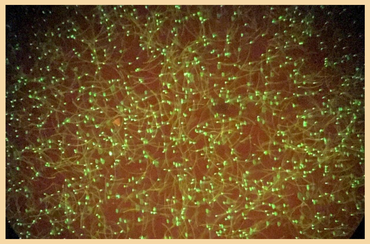
Samples mixed with Dimethyl Sulfoxide (DMSO) then field concentrations of glyphosate to test LD_{50} and LT_{50}

Figure 1: Drone bee

Collected samples stained with *Cybr*14 and propidium Iodide to differentiate Live/Dead .

Spermatozoa preparation on a hemocytometer was assessed using a fluorescent microscope. Live spermatozoa were observed with an excitation filter of 460-490nm (Green Fluorescent Protein, GFP) and a barrier filter of 510nm. Dead spermatozoa were observed with an excitation filter of 520-550nm (Red Fluorescent protein, RFP) and a barrier filter of 580nm.

Figure 2: GFP filter view of control sample showing live spermatozoa



Totals and proportion dead were calculated, probit analysis (R Core Team, 2013)

Results

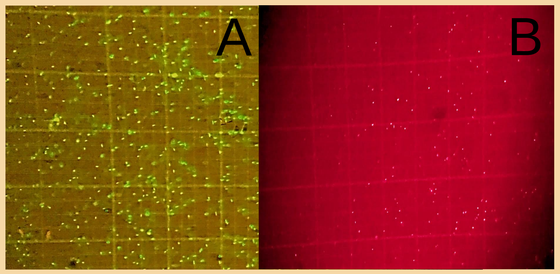


Figure 3: (A)GFP filter view of hemocytometer for counting. (B) RFP filter view of hemocytometer for counting

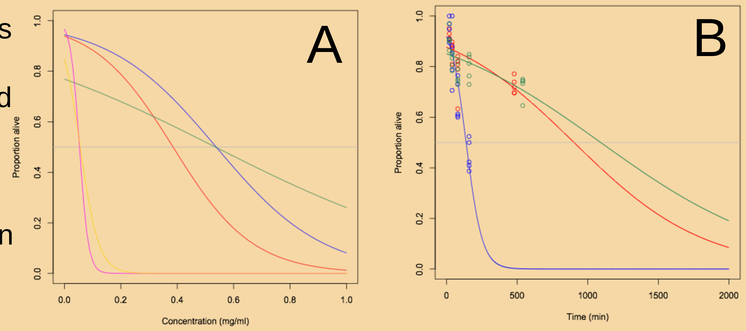


Figure 4: Probit analysis done using R, (A) showing proportion dead over concentration, LD50=0.07mg/mL, p=0.000 for 160 min of treatment. (B) Proportion dead over time, LT50=140 minutes, p=0.009 for 0.05mg/mL.

4A: red-20min, blue-40min, green-80min, magenta-160min, gold-480min
4B: red-0.025, blue-0.05, green-0.075

Conclusions

- DMSO, commonly used as a pesticide solvent, was tested and was not found to have any significant toxicity effects on spermatozoa.
- Monsanto reports that the LD50 for their product Roundup 3000® that contains 30% active glyphosate is at least 1mg/mL/bee. This study found the LD50 to be 0.07mg/mL for spermatozoa.
- The study supports the hypothesis that glyphosate does change reproductive cells. The data shows significant increase in the proportion of dead spermatozoa with both the increase in the concentration of glyphosate (p=0.000) and time of exposure (p=0.009).

Future Directions

- More research will be conducted to determine how glyphosate kills the cells via TUNEL assay (DNA breakage), single cell gel electrophoresis (DNA fragmentation) and mitochondrial viability staining.
- The study will be extended to other classes of common pesticides such as neonicotinoids (eg. Imidacloprid). This will alleviate the lack of knowledge of the direct effects of commonly used pesticides on the male honeybee.

Acknowledgments

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