The Development of a Free-Flying Method To Study the Effect of Neonicotinoids on the Positive Transfer of Learning in the Visual Domain of Honey Bees

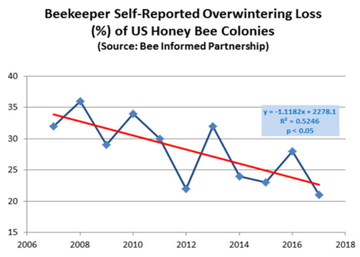
Research Background

Earth's Most Predominant Pollinators

As of 2020, 87 leading crops are dependent on honey bees, with the dollar value of these crops being $657 billion per year

Insecticides Are A Detrimental Factor

In a global survey of 198 honey samples, 75% contained at least one neonicotinoid, one of the most widely used insecticides worldwide with many containing Hazard Quotients above 1000.



Graph taken from: Genetic Literacy Project, 2023

THE ENVIRONMENTAL CRISIS

The Earth's most crucial pollinators face a grim future, as honey bee populations continue to decline, devastating our economies & ecosystems.

Objectives

1. Identify the effect of varying sublethal doses of neonicotinoids imidacloprid and clothianidin on the ability of honey bees to learn a **delayed matching to sample (DMTS)** rule followed by a **positive transfer of learning test** in their visual domain
2. Identify the specific memory **phases** (long, medium, short) associated with training and testing
3. Develop a method to train and test bees in a completely **open** environment, simulating a **real-world setting** and eliminating external stressors throughout experimentation

HYPOTHESIS

If bees consume neonicotinoids (imidacloprid, clothianidin), their ability to perform positive transfer in their visual domain will decrease.

Methodology

Experimental Procedure Overview

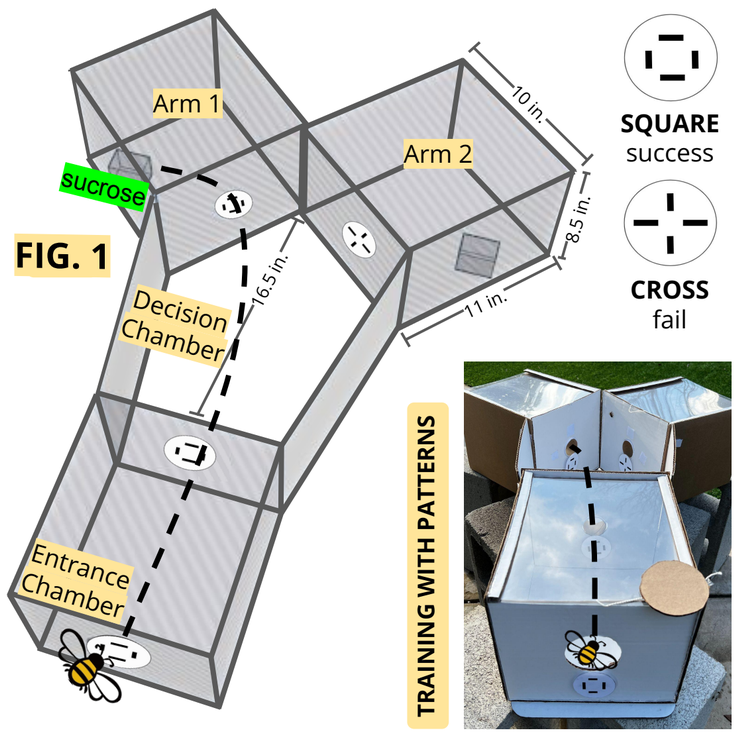
STEP 1
- Literature Review
- Design of Experiment
- Purchase Bee Hives + Materials
- Design of Maze
- Getting Familiar with Bees

STEP 2
- Initial setup of maze in my backyard
- Familiarity Test #1, #2, #3 with color, patterns, and y-maze

STEP 3
- Experiment #0: Training + Testing with Control Group
- Prepare Imidacloprid and Clothianidin Solution

STEP 4
- Experiment #1: Training + Testing with (a) 10nm and (b) 100 nm imidacloprid
- Experiment #2: Repeat Exp. #2 with clothianidin

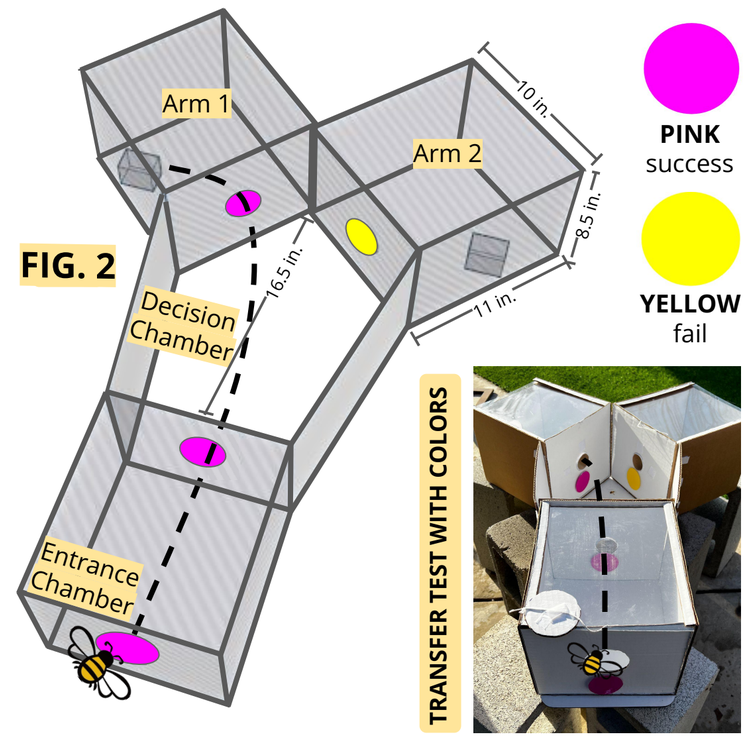
Delayed Matching to Sample Rule



Delayed Matching to Sample: honey bees must associate sample stimulus at the entrance chamber with secondary stimulus at the decision chamber, a test of their higher-order cognitive abilities

Example: In Fig. 1, bees must enter Arm 1, as it contains the square pattern, which matches with the pattern at the entrance chamber

Positive Transfer of Learning

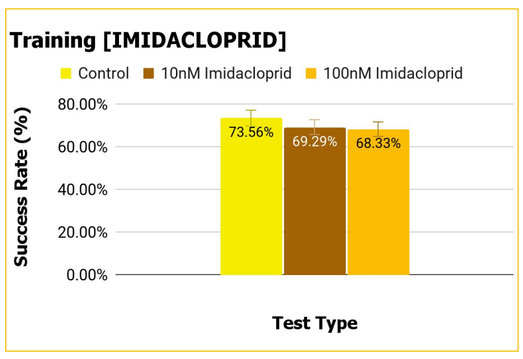


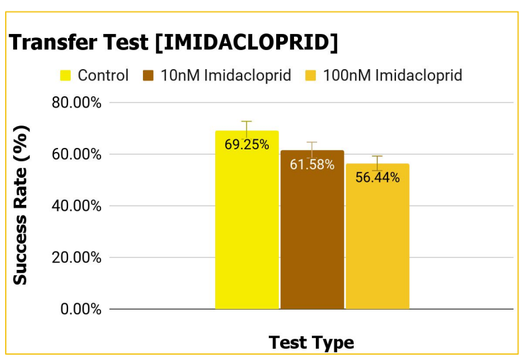
Positive Transfer of Learning: honey bees must transfer the DMTS rule from patterns (used in training) to colors (used in testing), simulating realistic information processing tasks utilized in foraging

Example: In Fig. 2, without having interacted with color stimuli prior, bees must enter Arm 1 to successfully complete positive transfer of DMTS

Results & Discussion

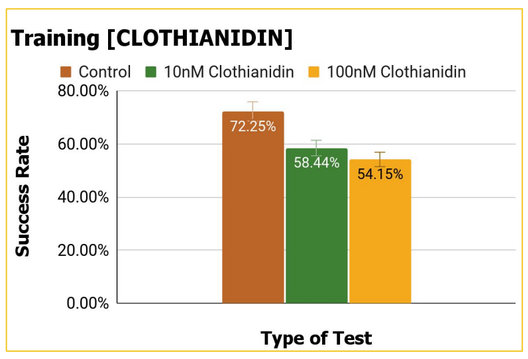
Experiment Phase 1: Imidacloprid





Imidacloprid had a **negative** and statistically significant effect ($p<0.05$) on positive transfer of learning at 10 nM, with an **extreme negative** effect at 100 nM.

Experiment Phase 2: Clothianidin



Clothianidin had an **extreme negative** and statistically significant ($p<0.05$) effect on positive transfer of learning at **both** 10 nM and 100 nM dosages.

- Compared to the control, the training success rates were not statistically significant ($p>0.05$), indicating that LTM was not impaired as bees successfully completed DMTS with patterns.
- As the honey bee completes transfer tests with colors (not stored in LTM), they must go through the MTM consolidation process again, which in this case is impaired due to the statistically significant performance ($p<0.05$) when compared to the control.
- In both training and testing, honey bees had decreased success rates compared to the control, indicating an inability to retrieve the DMTS rule when presented with both pattern and color stimuli. This suggests a loss of long-term, and likely both short- and medium-term memory.
- Clothianidin proved to be the more harmful insecticide when compared to imidacloprid

All images and graphs created by student researcher unless otherwise stated

Significance of Research

1. This research indicates the detrimental effects of neonicotinoids on honey bee higher-order cognition, with results extending to foraging abilities. Its findings serve as a call to decrease the usage of pesticides worldwide.
2. This research has established an innovative free-flying method, which can be applied to a multitude of other conditioning studies to simulate realistic experimental settings.
3. This research extends beyond current studies and analyzes the honey bee's higher-order cognitive abilities through positive transfer of learning, creating a task more similar to the complexity of honey bee real-world functions.