Lakxshanna Raveendran
Soyoun Moon

Research Plan

The Effect of Sulfur Dioxide on the Olfactory Learning of Western Honeybees (*Apis mellifera*).

A. Rationale:

Air pollution has become a major concern as humans continue to pollute the Earth with increasing amounts of industrial waste. The burning of fossil fuels results in the production of pollutants such as sulfur dioxide (SO_2). Sulfur dioxide is extremely dangerous and is completely man-made. The levels of SO_2 correlate directly with human activity. Pollution in the habitats of species which are already declining in population can be especially alarming as this can support the rise of endangered populations. The increase in air pollution poses a threat to the population of Western Honeybees (*Apis mellifera*), as studies show that air pollutants reduce their ability to perceive floral volatiles, preventing pollination (Ginevan et al., 1980). Honeybees use airborne pheromones to find their food sources such as nectar and pollen. Pollutants disrupt this process, as they deteriorate these pheromones thereby increasing difficulty of finding flowers to pollinate. These pheromones also help honeybees to distinguish previously depleted flowers to maximize foraging efficiency (Slessor et al., 2005). Therefore, as air pollutants degenerate these pheromones and floral volatiles, honeybees may be disoriented. About 73% of cultivated crop varieties are pollinated by bees, (Abrol 2012). As a result, it is imperative to research the effects of potentially harmful pollutants such SO_2 to honeybees in order to gain knowledge of their dangers and understand how the issue of the honeybee decline can be addressed.

B. Hypotheses, Research Questions and Expected Outcomes:

Does air pollution in the form of sulfur dioxide have an effect on the olfactory learning of Western Honeybees (*Apis mellifera*)?

We hypothesize that if Honeybees are exposed to SO_2 before classical conditioning it will take them longer to learn. We also hypothesize that if Honeybees are exposed to SO_2 after classical conditioning with lavender, the honeybees will no longer extend their proboscis as SO_2 will impact their olfactory memory (Bitterman et al., 1983).

C. Research Methods

Classical Conditioning (Learning)

- Freeze the honeybee for 5 minutes. Leave it in the freezer for an additional 1-2 minutes if the bee is still active.
- Harness the bee in a modified bullet using a thin piece of tape. Let the bee's head hang over the edge of the bullet tip and allow the thorax to rest on the inner ledge. Allow for the recovery of bees.
- Expose the honeybee to 2 mL of lavender oil. Put the 2 mL of lavender oil on a strip of filter paper. Secure the filter paper to the plunger seal of a syringe using a thumbtack. Push the plunger of the syringe inwards, expelling air towards the bee.
- Follow up the lavender oil directly with a 10% sucrose solution. Apply 2 mL of the solution on the tip of a Q-tip. Then gently tap the antennae of the honeybee with the Q-tip.
- Conduct a PER Assay

Proboscis Extension Reflex (PER) Assay

- Observe and score the extension of the proboscis. The bee can be considered fully conditioned when it repeatedly extends its proboscis after lavender exposure throughout 85% of the trials.
- If the honeybee extends its proboscis during the lavender but before the sucrose a '1' will be recorded. If the proboscis does not extend to the lavender '0' will be recorded.

Creating Sulfur Dioxide

- The following reaction will be used to create sulfur dioxide:

$$Na_2SO_3 + H_2SO_4 \rightarrow Na_2SO_4 + H_2O + SO_2$$

- Add 5 mL of sulfuric acid to the pressure-equalizing dropping addition funnel.
- Cover the bottom of a 2 necked round bottom flask with 10 grams of sodium sulfite.
- Slowly drip in the sulfuric acid by slightly opening the stopcock's flow of the pressure-equalizing dropping addition funnel.
- Once a bubbling reaction begins, open the stopcock connected to the main flask and rubber hose. A total of 0.03 moles of sulfur dioxide should be produced.

Experimental group

- Expose the honeybee to 6M dry sulfur dioxide gas for approximately 3 seconds.
- After exposure conduct classical conditioning procedure along with PER assays 15 times.

Control group

- Conduct classical conditioning procedure along with PER assays 15 times.

Risk and Safety

- Sulfur Dioxide causes severe skin burns and eye damage, toxic if inhaled.
- Work under a fume hood, wear gloves, goggles, and an apron.
- Be cautious when handling honeybees, as they have the potential to sting.
- Disposal: Allow sulfur dioxide to burn off. Any residual waste should be returned to the waste container.

Data Analysis

- PER Assay results will be statistical analyzed for control and experimental groups

Computational Analysis

- Import National Emissions Inventory (NEI) source type data as a CSV (comma separated value) file.
- Import libraries including: NumPy, Pandas, and Plotly.tools.
- Create empty arrays for black carbon, sulfur dioxide, nitrates, and carbon monoxide, and FIPS County number.
- Create a for loop such that as the program reads through the data, for each county in New York, the total emission tons of black carbon, sulfur dioxide, nitrates, and carbon monoxide from mobile source types will be added. The sum of these separate mobile source type pollutant emissions will represent the amount of diesel exhaust (tons) emit per year in each county.

FUTURE RESEARCH & LIMITATIONS

For future studies, we could include the use of electroantennograms to determine if the impairment that pollution causes directly impacts the antennae or the brain, as we are uncertain as to the location of where the olfaction system was affected. It would also be practical to determine if other pollutants that are abundant in our atmosphere impacts olfactory learning and how source type plays a factor. Additionally, seeing as we only focused on the urban, industrial areas of New York, the difference of floral perception among urban, suburban and rural areas could be investigated. Considering our methodology of exposing the honeybees to sulfur dioxide were not precise, replicating this objective with a fixed amount of sulfur dioxide released into the bees' closed space, would produce more exact results.

BIBLIOGRAPHY

Abrol, D. P. (2012). Decline in pollinators. In *Pollination Biology* (pp. 545-601). Springer, Dordrecht.

Bitterman, M. E., Menzel, R., Fietz, A., & Schäfer, S. (1983). Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). *Journal of Comparative Psychology, 97* (2), 107.

Fuentes, J. D., Chamecki, M., Roulston, T. A., Chen, B., & Pratt, K. R. (2016). Air pollutants degrade floral scents and increase insect foraging times. *Atmospheric Environment, 141*, 361-374.

Ginevan, M. E., Lane, D. D., & Greenberg, L. (1980). Ambient air concentration of sulfur dioxide affects flight activity in bees. *Proceedings of the National Academy of Sciences,* 77(10), 5631-5633.

Jiang, L., Xu, Y. S., & Ding, A. Z. (2010). Reaction of stabilized Criegee intermediates from ozonolysis of limonene with sulfur dioxide: ab initio and DFT study. *The Journal of Physical Chemistry A*, *114*(47), 12452-12461.

Le Conte, Y., & Navajas, M. (2008). Climate change: impact on honey bee populations and diseases. *Revue Scientifique et Technique-Office International des Epizooties*, *27*(2), 499-510.

Lusebrink, I., Girling, R. D., Farthing, E., Newman, T. A., Jackson, C. W., & Poppy, G. M. (2015). The effects of diesel exhaust pollution on floral volatiles and the consequences for honey bee olfaction. *Journal of chemical ecology*, *41*(10), 904-912.

Nieh, J. C., Tautz, J., Spaethe, J., & Bartareau, T. (2000). The communication of food location by a primitive stingless bee. *Zoology*, *102*(1999), 238-246.

Pankiw, T., Page Jr, R. E., & Fondrk, M. K. (1998). Brood pheromone stimulates pollen foraging in honey bees (*Apis mellifera*). *Behavioral Ecology and Sociobiology*, *44*(3), 193-198.

Potter, A., Andersson, J., Sjöblom, A., Junedahl, E., Palm Cousins, A., Brorström-Lundén, E., & Cato, I. (2005). Results from the Swedish National Screening Programme 2004: Subreport 3: Limonene.

Slessor, K. N., Winston, M. L., & Le Conte, Y. (2005). Pheromone communication in the honeybee (*Apis mellifera* L.). *Journal of Chemical Ecology*, *31*(11), 2731-2745.

Stout, J. C., & Goulson, D. (2001). The use of conspecific and interspecific scent marks by foraging bumblebees and honeybees. *Animal Behaviour*, *62*(1), 183-189.

NO ADDENDUMS EXIST