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Analysis of the Effect of the Herbicide, Glyphosate, on Parkinson's Disease Related Gene Expression in *Caenorhabditis elegans* and *Drosophila melanogaster*

Research Plan

A. Rationale:

Herbicides and pesticides are widely used across the world as a tool for harvesting food sources for humans to eat. The agricultural revolution had substantial impact on the world and led to many people settling into cities that developed into the ones we live in today. Therefore, it is imminent to investigate and analyze if these modern herbicides are safe to use, come in contact with, and ultimately ingest. This experiment addresses the issues of haphazardly using herbicides, such as Glyphosate, found in common products such as *Roundup* in household gardens and lawns. Many have found that this act has led to them becoming very sick and becoming prone to developing cancer. Therefore, the purpose of this experiment is to investigate the effects of Glyphosate on Parkinson's Disease (PD) related gene expression in *Caenorhabditis elegans* and *Drosophila melanogaster*. The procedure used in this experiment also serve to develop methods for testing environmental factors on other neurological diseases to better understand what humans should or should not expose themselves too.

An herbicide is a chemical agent used to destroy or inhibit plant growth. During the Vietnam War, an herbicide called Agent Orange was used to defoliate the battle grounds. However, in later years, it was found that veterans who fought in the Vietnam War developed Parkinson's Disease ("Environmental Factors" 2017). Now, the latest herbicide is Glyphosate in *Roundup*, which can possibly be linked to the development of illness. Glyphosate is a non-selective herbicide and will kill all plants it comes in contact with. Exposure to Glyphosate can be caused by physical contact or inhalation perhaps if a plant was recently sprayed with the herbicide. In a real world perspective, it is important to acknowledge a case from August 2017 involving a school groundskeeper, Dewayne Johnson, who sued Monsanto, the company that manufacturing herbicides like Roundup Brand, claiming that the use of the weed killer caused him to develop terminal cancer (Bellon 2018). In more recent news, *RoundUp* has begun to be removed from shelves for testing and toxin analysis. In 2015, it was discovered that Glyphosate was "probably carcinogenic to humans," according to the World Health Organization (WHO). Therefore, it is import to handle it with proper laboratory safety procedures such as wearing gloves, face masks, and goggles. Spraying the product in the fume hood is another safety precaution taken in accordance to Biosafety Level 1 (BSL1) rules. This uncertainty from the WHO is uncomfortable and provides incentive for studying this issue.

B. Research Questions

The main purpose of this study was to investigate the effects of the herbicide, Glyphosate, in the form of the weed killer *Roundup*, on the gene expression of Parkinson's Disease in *C. elegans*. By using the model organisms, *C. elegans* and *D. melanogaster*, a DNA analysis can be conducted to see if either the **PINK1** or **PARK7** gene is amplified. *D. melanogaster* genes can be analyzed to see if the **LRRK2** gene is amplified in the presence of Glyphosate. A secondary portion of this experiment would be to find a statistical correlation between increased frequency of Parkinson's Disease in the United States and sale of Glyphosate-related products. Therefore, it was hypothesized that when *C. elegans* are exposed to

the 0.75 milliliters or 1.00 milliliters of the weed killer, *Roundup*, with a 2% concentration of glyphosate in their medium, the genes PINK1 or PARK7 will be amplified more than in *C. elegans* that were not exposed to *Roundup*. Similarly, when *D. melanogaster* are exposed the weed killer, their expression of the LRRK2 gene will be amplified when compared to *D. melanogaster* that were not exposed to the substance. The utilization of two different amounts of *RoundUp* is meant to be synonymous to the varying amounts of weed killer used by each user as there are no specific instructions, which is another major issue.

C. Procedures

Caenorhabditis elegans are a nematode worm that are used in a laboratory setting as a model organism due to their short life span and short reproductive cycle which is advantageous over vertebrate organisms. The *C. elegans* genome is 100 million base pairs in length and contains a similar number of genes as humans, about 20,500 genes (“Why use the worm?” 2015). They can be utilized in microbiology because they provide examples of how neurological disorders, such as Parkinson’s Disease works in humans. Comparatively, *D. melanogaster* are a small model organisms used in a laboratory setting as a model organism due to their short life span and reproductive cycle. Their genome has over 100,000,000 base pairs with 75% of their disease causing genes similar to humans. They eat a simple diet and can be readily maintained in the laboratory setting which makes them useful in disease studies and how the susceptibility of diseases can be similar to humans.

Parkinson’s Disease is a progressive disorder of the body’s nervous system that heavily affects movement. Many definite causes are unknown and there is currently no cure for the disease (“What Is Parkinson’s?” 2018). PD entails the targeting of dopamine-producing or dopaminergic neurons in the brain. It begins as a tremor in a limb of the body, most commonly a tremor in the hand. The disease is caused by genetic factors and limited testing is being conducted to see if environmental factors also causes or progresses it. *C. elegans* and *D. melanogaster* have genetic homologies for PD and can be used to test its effects. By using, the N2, wild, strain of *C. elegans*, one of the genes involved in the development of the disease, LRRK2 (Cooper & Raamsdonk 2018), can tested for amplification, after the *C. elegans* are exposed to a minute dosage of weed killer in their cultures, The SNCA and PARK7 genes are also genetic markers for the disease. The exposed *C. elegans* can be monitored under a microscope and observed genetically through a gel electrophoresis and polymerase chain reaction (PCR). Based on this research it was hypothesized that if *C. elegans* are exposed to Glyphosate in the weed killer, *Roundup*, then the expression of the LRRK2 gene for Parkinson’s Disease will be amplified and visible in a DNA fingerprint. The same methods, including RNA extraction by Trizol protocol, were used in analysis of the *D. melanogaster* genomes. The end goal of this experiment is to show if Glyphosate is a definitive cause of PD or amplifies its gene expression. This could provide substantial evidence to stop the use of herbicides in agriculture and switch to safer alternatives.

D. Bibliography

- Abnova. YouTube, YouTube, 6 Jan. 2011, www.youtube.com/watch?v=RmPsLoIPRwc
- Why use the worm in research? (2015, June 19). Retrieved August 15, 2018, from <https://www.yourgenome.org/facts/why-use-the-worm-in-research>
- Bellon, T. (2018, August 07). First trial alleging Monsanto's Roundup causes cancer goes to jury. Retrieved September 4, 2018, from <https://www.reuters.com/article/us-monsanto-cancer-lawsuit/first-trial-alleging-monsanto-s-roundup-causes-cancer-goes-to-jury-idUSKBN1KS2G8>
- Bellon, T. (2018, August 11). Monsanto ordered to pay \$289 million in world's first Roundup... Retrieved September 4, 2018, from <https://www.reuters.com/article/us-monsanto-cancer-lawsuit/monsanto-ordered-to-pay-289-million-in-worlds-first-roundup-cancer-trial-idUSKBN1KV2HB>
- Cooper, J. F., & Raamsdonk, J. M. (2018, February 17). Modeling Parkinson's Disease in *C. elegans*. Retrieved September 4, 2018, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5836411/>
- Environmental Factors. (2017, October 17). Retrieved September 1, 2018, from <http://parkinson.org/Understanding-Parkinsons/Causes-and-Statistics/Environmental-Factors>
- Gao, L., Gómez-Garre, P., Díaz-Corrales, F. J., Carrillo, F., Carballo, M., Palomino, A., . . . Mir, P. (2009, August). Prevalence and clinical features of LRRK2 mutations in patients with Parkinson's disease. *Journal of Parkinson's Disease*, 3(4), 401-408. Retrieved July 2014, from www.researchgate.net/figure/Life-cycle-of-C-elegans-C-elegans-hermaphrodite-life-cycle-is-dependent-on_fig2_279947971
- Malkan, S. (2019, September 6). Glyphosate Fact Sheet: Cancer and Other Health Concerns. Retrieved from <https://usrtk.org/pesticides/glyphosate-health-concerns/>
- Martinez, Bryan A, et al. "C. Elegans as a Model System to Accelerate Discovery for Parkinson Disease." *Journal of Parkinson's Disease*. ScienceDirect, 2017. wormshack.ua.edu/uploads/8/7/2/5/87258178/martinez-rev-cogd-2017.pdf
- Parkinson's disease. (2018, June 30). Retrieved August 15, 2018, from <https://www.mayoclinic.org/diseases-conditions/parkinsons-disease/symptoms-causes/syc-20376055>
- Parkinsonian Worms May Hold the Key to Identifying Drugs for Parkinson's Disease. (2011, November 10). Retrieved September 4, 2018, from <https://neurosciencenews.com/parkinsonian-worms-parkinson-s-disease-drugs/>
- What Is Parkinson's? (2018, August 10). Retrieved September 1, 2018, from <http://www.parkinson.org/understanding-parkinsons/what-is-parkinsons>

