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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 Paper

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 to 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 uncomforting and provides incentive for studying this issue.

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 in 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.

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. The 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.

Overall, the DNA fingerprints showed that the rtPCR worked for a few samples as the gene primer bands were visible. This means that the program inputted into the thermal cycler can be utilized again in the future to obtain better results. Although, this experiment did not rule herbicides as a definitive cause for Parkinson's Disease, it can be inferred that with more studies this can be found it. Lastly, by improving on possible sources of error, a better concentration of RNA can be extracted to use in the rtPCR process in testing for amplified genes.